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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society and the Association of Public Analysts, organised by the Western Section of the Society, was held at 2.30 p.m. on Saturday, December 10th, 1955, in the Council Chambers of the City Hall, Cardiff. Members of the Food Group of the Society of Chemical Industry were invited to attend.

In the absence through illness of the Lord Mayor of Cardiff, the Deputy Lord Mayor, accompanied by the Deputy Lady Mayoress, gave a short address of welcome to the 50 members and visitors who were present.

Dr. K. A. Williams, F.R.I.C., A.Inst.P., M.Inst.Pet., President of the Society, invited Mr. T. McLachlan, D.C.M., A.C.G.F.C., F.R.I.C., President of the Association of Public Analysts, to take the Chair, and the following paper was presented and discussed: "Sucrose Loss from Ice-cream on Storage," by H. J. Evans, B.Sc., F.R.I.C., W. Kwantes, M.A., M.B., B.Chir., Dip.Bact., D. C. Jenkins, B.Sc., M.Sc., F.R.I.C., and J. I. Phillips, F.R.I.C.

NEW MEMBERS

ORDINARY MEMBERS

Robert Ballantyne Adamson, A.R.I.C.; Guy Houghton Baker, A.R.I.C.; Herbert Buchwald, B.Sc. (Lond.); Doreen Marjorie Henney; George Hunter, M.A., D.Sc., F.R.S.C.; John Alexander Hunter, B.Sc., Ph.D. (Edin.); Sidney Bertram Phillips, F.R.I.C.; Peter Sleightholm, M.A., B.Sc. (Oxon.), A.R.I.C.; Tsugio Takeuchi, Dr.Ing. (Tokyo); Eric Ernest Taylor, B.Sc. (Lond.).

JUNIOR MEMBER

John Barry Trueman, B.A. (Oxon.).

DEATHS

WE record with regret the deaths of

Francis Hereward Burstall
Edgar Wilfrid Deag
Thomas Francis Doyle
Thomas Shortridge Tweedie.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Wednesday, December 14th, 1955, in the George Hotel, George Street, Edinburgh. The Chair was taken by the Chairman of the Section, Dr. F. J. Elliott, F.R.I.C., F.R.S.E.

A lecture entitled "Statistics for Chemists (Statistical Control in Chemical Analysis)" was given by B. Woolf, M.A., Ph.D., F.R.S.E.

WESTERN SECTION

THE First Annual General Meeting of the Section was held at 11.30 a.m. on Saturday, December 10th, 1955, at the Park Hotel, Cardiff. Mr. H. J. Evans, B.Sc., F.R.I.C., was in the Chair. The following Officers and Committee Members were elected for the forthcoming years:—*Chairman*—Mr. P. J. C. Heywood. *Vice-Chairman*—Mr. R. H. Ellis. *Hon.*

Secretary and Treasurer—Dr. G. V. James, Western Counties Laboratory, 45 Colston Street, Bristol, 1. *Members of Committee*—Messrs. S. Dixon, A. Dobbins, H. J. Evans, R. H. Jones, T. G. Morris and R. Stephens. Dr. Z. Hybs and Mr. A. Richards were re-appointed as Hon. Auditors.

The Annual General Meeting was followed at 2.30 p.m., in the Council Chambers of the City Hall, by a Joint Meeting of the Society with the Association of Public Analysts, organised by the Western Section. The following paper was presented and discussed: "Sucrose Loss from Ice-cream on Storage," by H. J. Evans, B.Sc., F.R.I.C., W. Kwantes, M.A., M.B., B.Chir., Dip.Bact., D. C. Jenkins, B.Sc., M.Sc., F.R.I.C., and J. I. Phillips, F.R.I.C.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, December 7th, 1955, at the Mason Lecture Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

A paper on "Some Physical Methods for the Analysis of Phosphorus Compounds" was given by Dr. D. E. C. Corbridge.

An Ordinary Meeting of the Section was held at 7 p.m. on Thursday, December 15th, 1955, at the Technical College, Nottingham. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

A lecture was given on "Recent Advances in Inorganic Analysis" by R. Belcher, Ph.D., F.R.I.C., F.Inst.F.

PHYSICAL METHODS GROUP AND WESTERN SECTION

The 51st Ordinary Meeting of the Physical Methods Group was held jointly with the Western Section at 6.30 p.m. on Friday, October 28th, 1955, in the Chemistry Department, Woodland Road Entrance, Bristol University. The Chair was taken by Dr. J. E. Page.

The following papers were presented and discussed: "X-ray Analysis of the Structure of Vitamin B_{12}," by Dorothy Crowfoot Hodgkin, B.Sc., M.A., Ph.D., F.R.S. (see summary below); "X-ray Fluorescent Quantitative Analysis as a Tool in Archaeology," by E. T. Hall, M.A., D.Phil.; "X-ray Diffraction Techniques in the Investigation of Crime," by E. B. Parkes, M.Sc., F.R.I.C.

Visits were made before the meeting to John Harvey's Cellars and to the Long Ashton Research Station.

The X-ray Analysis of the Structure of Vitamin B_{12}

Dr. Hodgkin said that, since the isolation of crystalline vitamin B_{12} seven years ago, they had used X-ray diffraction methods to assist in finding the chemical structure of the vitamin. Their examination had been closely interlocked with chemical investigations carried out in a number of laboratories. These showed that the vitamin had approximately the formula $C_{61-64}H_{83-92}N_{14}O_{13-14}PCo$ and that it contained a CN group, a nucleotide-like group built of benziminazole, ribose and phosphate units, a propanolamine residue and probably six amide groups attached to a large remaining nucleus of unknown structure containing about 45 carbon atoms.

They had found it possible to discover the arrangement of the atoms in this nucleus through the calculation of the electron-density distribution for four different crystals: air-dried and wet vitamin-B_{12} crystals, vitamin-B_{12}.SeCN, and the hexacarboxylic acid degradation product of vitamin B_{12} prepared by Cannon, Johnson and Todd. In these calculations they had used phases for the terms in the Fourier series employed based on, first, the cobalt atoms, and then on gradually increasing known regions of the structure; the correct atomic positions could be selected from among the many spurious peaks that appeared in the first approximate calculated distributions. The whole project had required very heavy computing, which had been carried out partly in this country, partly in America.

They could now write a tentative complete structure for the vitamin. The inner nucleus had been found to have interesting and unexpected structural relations with porphyrins. The molecule as a whole was very compact; a number of small chemical

modifications in its structure could be made without changing its ability to form crystals with essentially similar molecular arrangements.

BIOLOGICAL METHODS GROUP

THE Eleventh Annual General Meeting of the Group was held in the meeting room of the Chemical Society, Burlington House, London, W.1, on Friday, December 9th, 1955, at 6.15 p.m. The Chair was taken by Dr. L. J. Harris, F.R.I.C. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Mr. K. L. Smith. *Vice-Chairman*—Mr. S. A. Price. *Hon. Secretary and Treasurer*—Mr. K. A. Lees, Glaxo Laboratories Ltd., Sefton Park, Stoke Poges, Bucks. *Members of Committee*—Dr. L. J. Harris, Miss A. M. Jones, Drs. J. I. M. Jones, S. K. Kon, K. C. Sellers and E. C. Wood. Mr. J. W. Lightbown was re-appointed Hon. Recorder, and Messrs. D. M. Freeland and J. H. Hamence were re-appointed as Hon. Auditors.

The Annual General Meeting was followed at 6.30 p.m. by an Ordinary Meeting of the Group. The following papers were presented and discussed: "The Microbiological Plate Assay of Penicillin in Compound Feeding Stuffs," by J. S. Simpson and K. A. Lees, F.P.S., D.B.A.; "A Simple Method for the Determination of pA_2 at 2 Minutes," by Mary F. Lockett, M.B., B.S., M.D., M.R.C.P., Ph.D.; and a demonstration of an automatic apparatus for isolated preparations, suitable for the assay of oxytocin and similar assays, was given by J. A. Lock, B.Sc., F.P.S. (see summary below).

AN AUTOMATIC APPARATUS FOR ISOLATED PREPARATIONS, SUITABLE FOR THE ASSAY OF OXYTOCIN AND SIMILAR ASSAYS

MR. LOCK demonstrated an apparatus* that was basically similar to those previously described, but which had the practical advantage that doses of drug were added directly to the isolated-organ bath by adjustable relay-controlled syringes.

This arrangement overcame the difficulty that frequently arose when the drug was incorporated in reservoirs of bathing fluid: trial doses added directly to the bath, when extrapolated to the necessary dilutions in the bathing fluid, were not always found to be effective, and the reservoir might have to be emptied and refilled several times before a satisfactory result was achieved. Further, the preparation might vary in sensitivity in the course of an assay; if this occurred the assay might have to be abandoned. In the arrangement demonstrated adjustments of doses could be carried out rapidly with no disturbance to the rest of the apparatus.

* Previously demonstrated at a meeting of the British Pharmacological Society at Leeds, January 7th, 1955.

The Titrimetric Determination of Sulphate with 4-Amino-4′-chlorodiphenyl Hydrochloride as Reagent

By R. BELCHER, A. J. NUTTEN, E. PARRY and W. I. STEPHEN

(*Presented at the meeting of the Midlands Section on Wednesday, May 25th,* 1955)

An examination has been made of the use of 4-amino-4′-chlorodiphenyl as a reagent for the titrimetric determination of sulphate. Conditions have been established to permit the determination of 25 to 100 mg of sulphate in an initial volume of 100 ml and 2·5 to 25 mg in an initial volume of 50 ml. When a short standing time is used, it is essential to maintain a minimum final concentration of reagent above 0·12 per cent. to ensure complete precipitation in these volumes.

Attempts have been made to eliminate the interference of phosphate by simple means, but the only satisfactory way was to precipitate it as magnesium ammonium phosphate and filter off the precipitate.

The possibility of using salts other than the hydrochloride as reagents has been examined, but none had any advantage.

The use of benzidine as a reagent for the titrimetric determination of sulphate is well known and has found many applications.[1] At first sight the method appears to be attractive, for it is more rapid than that in which barium sulphate is the final weighing form, or than either of the other two available gravimetric methods,[2,3] but the sensitivity of benzidine towards sulphate is poor and it is not possible to obtain highly accurate results. Large amounts of nitrate interfere, and many other ions tend to be co-precipitated. A number of amines related to benzidine has been prepared,[4] one of which, 4:4′-diaminotolane, has a greater sensitivity towards sulphate.[5] This reagent can be used for the titrimetric determination of sulphate, but is not satisfactory for amounts less than 10 mg.[6] Further, its synthesis is troublesome.

During the course of this work the properties of the sulphates of several mono-amines were examined. It was found that although the sulphate of 4-aminodiphenyl was more soluble than that of benzidine, less sulphate ion was actually left in solution, owing to the higher molecular weight of the precipitate. The sensitivity of this amine towards sulphate was low, so that its use as a reagent was precluded, but it became apparent that low sulphate solubility was not a property confined to *para* substituted diamines. Accordingly, several *p*-aminodiphenyl derivatives substituted in the vacant *para* position were prepared.[7] One of these, 4-amino-4′-chlorodiphenyl, possessed many of the characteristics of the ideal reagent and was therefore proposed for use in the determination of sulphate. It was later applied to the determination of sulphur in organic compounds containing nitrogen.[8]

It has been suggested[5] that co-planarity and co-linearity are essential characteristics for low sulphate solubility, but this has been shown only for diamines. It remains to investigate the influence of these factors on the solubility of mono-amine sulphates, which doubtless have different crystal structures, and this work is now proceeding. Compounds such as 4-amino-4′-chlorostilbene and 4-amino-4′-chlorotolane should provide useful information along these lines.

The ideal reagent of this type should possess the following properties—

(*a*) it should be easy to synthesise,
(*b*) it should be sensitive towards sulphate,
(*c*) it should not be subject to interference by nitrate ions,
(*d*) it should be recoverable, and
(*e*) its other salts should be appreciably soluble.

4-Amino-4′-chlorodiphenyl does not fulfil all these conditions, and our search for new reagents in this field will be continued. Amongst its disadvantages is its non-selectivity (it is less selective than the barium ion). The precipitate must be dispersed on a macerated paper-pulp pad and boiled in order to effect complete reaction with standard alkali and, owing

to the low solubility of the amine hydrochloride (the salt used as reagent), large volumes of reagent solution must be used.

Although the solubility in water of the dried amine sulphate is very low, that of the freshly precipitated material appears to be higher. However, it should be emphasised that solubility in water merely acts as a guide to the suitability of a reagent, for its sensitivity to the ion being determined is of far greater significance. 4-Amino-4′-chlorodiphenyl is far more sensitive towards sulphate than any other known reagent. It will produce a pronounced turbidity with 0·001 *N* sulphuric acid, whereas a 10 per cent. barium chloride solution, although having the advantage of greater concentration, has no immediate effect. It may well be that the reagent could be used for the turbidimetric determination of sulphate, but its success would depend on the establishment of conditions under which a stable suspension is produced.

It may be that a reagent with more favourable properties will never be forthcoming, or at least not for some considerable time. Hence it was considered desirable to make a more extended study of this reagent so that it might possibly find further applications.

THE PRESENT INVESTIGATION—

The original paper describing the new reagent was primarily concerned with the theoretical aspects of the sulphate solubilities of 4-amino-4′-chlorodiphenyl and its analogues. The possibilities of the reagent were only shown under more or less ideal conditions, and very small volumes of sulphate solution were used (not more than 25 ml). It remained to examine sulphate recoveries with volumes of solution more in keeping with those found in practice (*e.g.*, 25 to 1000 ml).

It is known that zinc, copper, magnesium, iron^{III}, chromium^{III}, calcium and the alkali metals do not interfere when present in moderate amounts. Aluminium forms an insoluble complex salt containing sulphate, but its interference can be overcome by the addition of tartaric acid. Phosphate, tellurite, selenate and oxalate interfere by forming insoluble salts with the reagent. Tellurate and selenite have no effect, and it was hoped to base a separation of selenium and tellurium on these observations, but unfortunately the amine tellurite and selenate are not sufficiently insoluble.

The most serious interference from a practical point of view is that of phosphate. Hence further attempts have been made to eliminate it, for the usefulness of the reagent would then be enhanced.

One of the disadvantages attaching to the use of 4-amino-4′-chlorodiphenyl hydrochloride as a precipitant is its sparing solubility. The advantages of a more soluble salt are too obvious to enumerate, and it was felt that an examination of some of the accessible, although less common, salts of 4-amino-4′-chlorodiphenyl might prove worth while.

The hydrochloride is now available commercially, but some samples contain an insoluble compound in amounts up to 10 per cent. We have identified this as 4:4′-dichlorodiphenyl. When present, it can be removed by filtering the warm solution, but care should be taken to ensure that the filtrate is then saturated with respect to the reagent.

EXPERIMENTAL

VOLUME OF TEST SOLUTION—

It was found possible to work with larger volumes of solution than those originally recommended. Amounts of sulphate between 20 and 100 mg can be satisfactorily determined in volumes of up to 100 ml, whilst for amounts of sulphate between 2·5 and 20 mg the volume may be as large as 50 ml. A selection of results is given in Table I.

VOLUME AND FINAL CONCENTRATION OF REAGENT—

In the original paper[7] it was recommended that at least a twofold excess of reagent should be added. This is still necessary, but a further condition must now be stressed because of the increased volumes used. Precipitation is quantitative provided that the concentration of the solution after precipitation of sulphate is greater than 0·12 per cent. with respect to the hydrochloride of 4-amino-4′-chlorodiphenyl. Accordingly, if a 0·24 per cent. solution of the reagent is used to precipitate sulphate, somewhat more than an equal volume of the reagent must be added to attain this critical final concentration. When a more concentrated solution of the reagent (0·48 per cent.) is used for larger amounts of sulphate, the appropriate volume of reagent solution should be added. In each case the excess of

reagent must be at least twofold; in some cases, because of the concentration requirements, the excess of reagent may be considerably greater than this. Two millilitres of reagent of known concentration were added to 1-ml amounts of sulphate solution of decreasing concentration. The point at which no turbidity appeared after 5 to 10 minutes was taken as indicating that the concentration of reagent was insufficient to effect precipitation. Fig. 1 illustrates the relationship between the final concentration of the solution with respect to

TABLE I

RECOVERIES OF SULPHATE

Sulphate precipitated from 50 ml of solution		Sulphate precipitated from 100 ml of solution	
Present, mg	Found, mg	Present, mg	Found, mg
2·70	2·67	10·08	10·05
2·92	2·91	12·08	12·09*
3·31	3·35	21·12	21·16
3·84	3·88	25·20	25·25
4·67	4·64	34·34	34·38
5·04	5·01	50·02	50·08
6·83	6·87	60·00	60·15*
7·27	7·29*	71·50	71·65
10·34	10·66	84·48	84·28
14·38	14·34*	92·53	92·69*
19·40	19·43	100·32	100·20

* A 0·48 per cent. solution of reagent heated to 40° C was used.

the reagent and the amount of sulphate left in solution after precipitation. It will be seen that, when the final concentration of the reagent is 0·12 per cent. or greater, the amount of sulphate left in solution is insignificant.

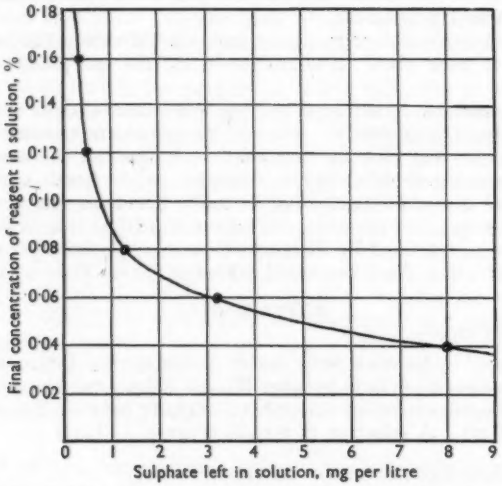

Fig. 1. Relationship between the final concentration of reagent in solution and the amount of sulphate left after precipitation

FILTRATION AND WASHING OF THE PRECIPITATE—

During the course of this work, it was found that if the pulp fibre used for the filtration had been prepared in a finely divided state, and a small amount of the pulp had previously been added to the precipitation vessel, the precipitate could be retained on a pad about $\frac{1}{8}$ to $\frac{1}{4}$ inch thick, irrespective of the amount of sulphate being determined. Washing was thus more easily carried out, because less reagent was held in the pad.

Washing with water alone yielded satisfactory results, but there is the possibility that over-washing of the precipitate may occur, especially in careless hands. This would lead to a slight negative error. A common device in analytical chemistry to avoid washing errors is to wash with a saturated solution of the precipitate itself. Accordingly, following a suggestion by Dr. B. B. Bauminger,[9] we have incorporated in our method a wash solution consisting of water saturated with 4-amino-4′-chlorodiphenyl sulphate. A larger blank value was then obtained when the pulp-pad method of filtration was used.

Other wash liquids tried were sodium acetate solution, potassium nitrate solution and aqueous ethanol, but none was satisfactory.

USE OF OTHER SALTS—

None of the salts of the base that was examined proved to be more soluble than the hydrochloride. In the present investigation the acetate, formate, propionate, oxalate, succinate, perchlorate, bromide, fluoride and iodide were prepared and examined.

REMOVAL OF PHOSPHATE—

It was hoped that it might be possible to precipitate phosphate by means of a suitable metal ion and then proceed with the determination in its presence. The only metal phosphate precipitated quantitatively under the conditions of the determination was that of bismuth. Unfortunately, this compound adsorbed appreciable amounts of sulphate and was therefore useless for our purpose. The only satisfactory method was to remove the phosphate as the magnesium ammonium salt (the phosphate could be determined in this precipitate if desired), separate the precipitate, adjust the pH to between 1 and 2 and then precipitate the sulphate. The methods used were as follows—

(*a*) A solution of bismuth perchlorate was added dropwise from a burette to a solution containing phosphate and sulphate until no further precipitation occurred. A few drops were added in excess. The sulphate was then precipitated and titrated in the usual way.

(*b*) Magnesia mixture was added to the slightly acid sulphate solution, 1 ml being added for each 10 mg of P_2O_5 present. Concentrated ammonia solution was then added, with stirring, until the mixture became slightly alkaline. One further millilitre of ammonia solution was added. The mixture was set aside for 1 hour at 0° C, and the precipitate was then filtered off and washed with dilute ammonia solution (1 + 19). The filtrate was boiled to expel ammonia, cooled, the pH was adjusted and the sulphate determined.

The results are shown in Table II.

TABLE II

REMOVAL OF PHOSPHATE

Phosphate present, mg	Sulphate present, mg	Sulphate found	
		Bismuth method, mg	Magnesium method, mg
30·00	30·00	27·3, 26·1	29·80, 29·7
15·00	30·00	28·0, 28·6	29·90, 29·80
30·00	25·00	—	25·1, 24·8
15·00	50·00	—	49·7, 49·9

METHOD

REAGENTS—

4-Amino-4′-chlorodiphenyl hydrochloride—A 0·24 per cent. solution in 0·05 *N* hydrochloric acid. Warm to dissolve the solid, filtering warm if necessary.

4-Amino-4′-chlorodiphenyl sulphate—A saturated aqueous solution.

Sodium hydroxide, 0·1 N, 0·05 N *and* 0·01 N.

Mixed-indicator solution—A mixture of 0·04 per cent. aqueous phenol red (sodium salt) and 0·04 per cent. aqueous bromothymol blue (sodium salt) in the ratio 3 to 2 by volume.

PROCEDURE FOR 2·5 TO 20 mg OF SULPHATE—

To a solution containing 2·5 to 20 mg of sulphate in a volume not greater than 50 ml and 0·05 *N* with respect to hydrochloric acid, add a little paper pulp and sufficient of a

0·24 per cent. solution of 4-amino-4′-chlorodiphenyl hydrochloride in 0·05 *N* hydrochloric acid to give at least a twofold excess and a final concentration above 0·12 per cent. Set aside for 20 to 30 minutes, stirring occasionally, and filter through a pulp pad, $\frac{1}{8}$ to $\frac{1}{4}$ inch deep. It is essential that this pad is prepared according to the method described by Belcher and Nutten.[10] Rinse the precipitation vessel with 5 to 10 ml of filtrate and transfer to the filter. Wash the precipitation vessel with two 3-ml portions of distilled water and then wash the precipitate and pad with a saturated aqueous solution of 4-amino-4′-chlorodiphenyl sulphate until the washings no longer give a test for chloride. Transfer the precipitate and pad to the original flask, stir vigorously with a glass rod to break up the precipitate, heat to boiling, add 3 to 4 drops of indicator and titrate with 0·01 *N* sodium hydroxide until a purple tinge appears. Re-heat and, if the colour changes, continue the titration until a permanent purple colour appears.

This procedure may be applied to the determination of sulphur in organic compounds on the micro or semi-micro scale after combustion of the sample and absorption of the combustion gases in hydrogen peroxide.

For the micro-determination, take a weight of sample so that not more than 2 mg of sulphur are present. The washings from the absorber must not exceed 50 ml. Stir in a small amount of paper pulp and add 70 ml of 0·24 per cent. reagent. Stir and set aside for 15 to 20 minutes and complete the determination as described above.

Alternatively, add 35 ml of 0·48 per cent. reagent heated to 40° C, cool under the tap, set aside for 15 to 20 minutes, and complete the determination as described above.

PROCEDURE FOR 20 TO 100 mg OF SULPHATE—

In this case the volume of sulphate solution should not exceed 100 ml and should be 0·05 *N* with respect to hydrochloric acid. Produce the precipitate as described above, filtering it off and washing it. Titrate the precipitate with 0·1 *N* or 0·05 *N* sodium hydroxide solution (depending on the amount of sulphate present).

If it is preferred to keep the total volume small, a 0·48 per cent. solution of the reagent in 0·05 *N* hydrochloric acid, heated to 40° C, may be used.

BLANK DETERMINATION—

Prepare the pad and wash with a few millilitres of saturated wash solution. Drain in the usual way and complete the titration as described above, using standard alkali of the appropriate concentration.

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DEPARTMENT OF CHEMISTRY
THE UNIVERSITY
EDGBASTON
BIRMINGHAM, 15

June 14th, 1955

The Use of 4-Amino-4′-chlorodiphenyl Hydrochloride for the Determination of Sulphur in Coal

By H. C. WILKINSON

(*Presented at the meeting of the Midlands Section on Wednesday, May 25th, 1955*)

It is shown that 4-amino-4′-chlorodiphenyl hydrochloride can be used for the determination of sulphur in coal by the bomb-combustion method. Results for a series of twelve coals covering a range of carbon content of approximately 83 to 93 per cent. (dry ash-free) show that the mean difference in sulphur content determined by using this reagent and by the Eschka method is 0·03 per cent. of sulphur. The time saved by the new method is approximately 35 minutes, and no special apparatus is necessary.

The determination of sulphur by combustion in a calorimeter bomb is a well established method, having found widespread favour on the grounds of its rapidity and accuracy. According to Strambi,[1] a determination of sulphur by this method may be made in about 1¼ hours, but, although Strambi has adopted many of the time-saving procedures suggested by Mott, Ruell and Wilkinson,[2] the time required for the precipitation, filtration and ignition of the barium sulphate is still large compared with the time of combustion of the sample.

Miller,[3] using barium rhodizonate as an internal indicator, and Radmacher,[4] using a modification of the chromate method of Andrews,[5] have attempted to shorten this period by using a titrimetric instead of a gravimetric finish, but these methods suffer from various disadvantages, such as the effect of nitrate ion. Belcher, Nutten and Stephen[6] have shown that the nitrate ion has no influence upon the precipitation of sulphur as the sulphate of 4-amino-4′-chlorodiphenyl and, as the effect of interfering ions present in coal ash, such as aluminium and iron, can be suppressed by making certain additions, trials of this new reagent were made.

There is no apparent effect due to interference by phosphorus or phosphates, probably because there is very little present, and not all would go into solution.

Initially, experiments were carried out with the fairly small volumes and the amounts of reagent originally recommended.[6] In a later communication,[7] it was demonstrated that larger volumes of solution could be used provided that certain precautions in the addition of the reagent were observed. Further, it was recommended that a wash solution saturated with the precipitate should be used to avoid any risk of overwashing. Although satisfactory results were obtained by using the original recommendations, the later conditions rendered the method more flexible, and were accordingly adopted. Since a pulp that would easily disintegrate on boiling was required for accuracy in the final titration, it was prepared from Whatman ashless tablets. These were found to be satisfactory in use, giving no difficulty from bumping on boiling.

It was also noted that there was an effect due to ferric hydroxide, the 4-amino-4′-chlorodiphenyl sulphate being oxidised to a purple compound, so leading to low results with coals of high pyritic sulphur (and consequently high iron) content, but tartaric acid was found to be effective in inhibiting this oxidation.

The method adopted for extracting the sulphur from the coal in the bomb was that put forward by Moszynski[8]: 1 g of coal was burned with 0·5 g of Eschka mixture in the bomb, which contained 5 ml of 10 per cent. ammonium carbonate, the oxygen pressure being 25 atmospheres. After firing, the pressure was released, the residue and the bomb being washed out with about 30 ml of hot distilled water. Boiling with 6 ml of concentrated hydrochloric acid dissolved any remaining residue. The pH of the solution was adjusted to about 1·4 by the addition of 4 ml of aqueous ammonia, sp.gr. 0·880, followed by 2·5 ml of concentrated hydrochloric acid, the total volume of the bomb washings being made up to 250 ml. After precipitation and washing, the sulphur was determined as recommended by Belcher *et al.*[7]

As the complete process takes 35 minutes, the time necessary for a determination of sulphur by a bomb-combustion method is reduced by about 30 minutes, but the accuracy

of the test is preserved. The 4-amino-4′-chlorodiphenyl may be recovered, as already described by Belcher *et al.*

Results for determinations on thirteen coals are shown in Table I, for a range of sulphur of 0·57 to 3·22 per cent.; comparative results by the Eschka method are also given, together with the mean algebraic and arithmetic differences and the difference between duplicate tests by the new method. The agreement between the two methods is good, showing that rapid determination of the sulphur in coal by a bomb-combustion method can be accurately carried out by using the 4-amino-4′-chlorodiphenyl reagent.

TABLE I

COMPARISON OF SULPHUR DETERMINATIONS BY ESCHKA (GRAVIMETRIC FINISH) AND BOMB-COMBUSTION (TITRIMETRIC FINISH) METHODS

Coal	Volatile matter (dry ash-free), %	Sulphur by: Eschka method (A), %	Sulphur by: Bomb-combustion method* (B), %	Difference (A–B), %	Difference between duplicate tests (B), %
S. Yorks.	39·0	3·22	3·10	+ 0·12	0·05
Leics.	39·7	2·36	2·33	+ 0·03	0·01
S. Yorks.	41·0	2·08	2·05	+ 0·03	0·00
Durham	33·3	1·99	2·03	− 0·04	0·01
Leics.	40·3	1·84	1·79	+ 0·05	0·02
S. Yorks.	38·3	1·45	1·45	± 0·00	0·04
Notts.	38·3	1·30	1·31	− 0·01	0·01
S. Yorks.	38·0	1·03	1·12	− 0·09	0·03
S. Yorks.	35·7	0·87	0·93	− 0·06	0·04
S. Wales	6·4	0·81	0·78	+ 0·03	0·03
Kent	18·6	0·70	0·67	+ 0·03	0·02
S. Wales	17·1	0·69	0·73	− 0·04	0·01
S. Wales	16·5	0·57	0·55	+ 0·02	0·02
Mean difference (A–B) for up to 2·5 per cent. of sulphur—arithmetic ..				0·032	0·02
Mean difference (A–B) for up to 2·5 per cent. of sulphur—algebraic ..				− 0·004	

* Bomb-combustion method with titrimetric finish, 4-amino-4′-chlorodiphenyl being used.

METHOD

REAGENTS—

4-Amino-4′-chlorodiphenyl hydrochloride, 0·48 per cent. solution—Dissolve 4·8 g of reagent in about 800 ml of hot distilled water containing 50 ml of *N* hydrochloric acid. Filter hot, and make up to 1 litre; keep the container securely stoppered to minimise effects due to oxidation.

Sodium hydroxide solution, standardised, approximately 0·1 N.

Mixed-indicator solution—A mixture of 0·04 per cent. aqueous phenol red (sodium salt) and 0·04 per cent. aqueous bromothymol blue (sodium salt) in the ratio of 3 to 2 by volume.

Tartaric acid solution—A solution containing 15 g of crystalline tartaric acid per litre of solution; 10 ml of this solution, as used in the test, will counteract the effect of aluminium and ferric iron, the concentration of the solution being based on average figures for the Al_2O_3 and Fe_2O_3 contents of normal British coals containing up to 10 per cent. of ash.

4-Amino-4′-chlorodiphenyl sulphate, saturated solution—Shake the sulphate with distilled water for 30 minutes, or, alternatively, allow excess of the solid to stand in contact with distilled water, topping up the solution as necessary.

PROCEDURE—

Make up the bomb-washings to 250 ml, as previously described. By pipette put 100 ml in a 400-ml conical beaker, and add a small amount of filter-paper pulp, together with 10 ml of the tartaric acid solution.

Add 60 ml of warm 0·48 per cent. 4-amino-4′-chlorodiphenyl hydrochloride solution and set the beaker aside for 20 minutes. A pulp pad consisting of two layers of fine

filter-paper pulp supported on a Witt-plate in a 60° funnel is used as the filtering medium, the total thickness of pulp being about $\frac{5}{16}$ inch. Entrapping of air-bubbles under the Witt-plate should be avoided when preparing the pad. Wash the precipitate on to the pad and rinse with 5 to 10 ml of distilled water, finally washing with small amounts of saturated 4-amino-4′-chlorodiphenyl sulphate solution until free from chloride.

Drain the filter by gentle suction, and remove the upper layer from the funnel by means of tweezers, using the lower layer to wipe out the funnel. This, together with the upper layer and Witt-plate, is transferred to the 400-ml conical beaker used for the initial precipitation.

Wash the funnel out into the beaker with 50 to 60 ml of distilled water, breaking up the aggregate of pulp and precipitate by vigorous stirring with a glass rod. It is important that the pulp should be thoroughly broken up, for the sodium hydroxide used for titration only reacts slowly with the precipitate, and a false value is obtained if the precipitate is masked by large aggregates of pulp.

Heat the contents of the beaker to boiling-point, boil for 1 minute, and titrate with the standard sodium hydroxide, using the mixed indicator. On observing the first purple coloration, re-heat the contents of the beaker, adding further sodium hydroxide solution until a permanent purple colour is observed.

CALCULATION—

1 ml of 0·10 *N* sodium hydroxide ≡ 4·8 mg of sulphate ≡ 1·6 mg of sulphur.
A 100-ml aliquot of bomb-washings contains the sulphur from 0·4 g of coal.

$$\therefore \text{ Sulphur in coal} = \frac{(\text{ml of NaOH}) \times 1{\cdot}6 \times 100}{0{\cdot}4 \times 1000} = (\text{ml of NaOH}) \times 0{\cdot}4 \text{ per cent.}$$

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June 9th, 1955

The Use of Vanadium Pentoxide and 4-Amino-4′-chlorodiphenyl Hydrochloride in the Combustion Method for the Determination of Total Sulphur in Rubber

By B. B. BAUMINGER

(*Presented at the meeting of the Midlands Section on Wednesday, May 25th*, 1955)

The determination of sulphur in organic and inorganic compounds and synthetic elastomers by combustion in an empty silica tube is described. Interference from alkaline-earth carbonate is eliminated, and sulphur in alkali and alkaline-earth sulphates is included, by treating with a catalyst mixture of vanadium pentoxide and zinc oxide at a temperature of 1000° C. Interference from chlorine and nitrogen is avoided by precipitating the sulphate with 4-amino-4′-chlorodiphenyl hydrochloride, the precipitate being filtered off, washed with a saturated solution of the amine sulphate and then titrated alkalimetrically.

The method in common use for the determination of total sulphur in natural and synthetic rubber compounds[1] has two main disadvantages—

(*a*) the necessity for high combustion temperatures (1350° C) for the analysis of rubber compounds containing calcium carbonate, barytes and similar materials, and

(*b*) the necessity for modified techniques when chlorine or nitrogen is present.

Conditions for the determination of total sulphur—

Decomposition of alkali and alkaline-earth sulphates to give a quantitative yield of sulphur oxides can be brought about at a temperature of 1000° C by treating the sample with a mixture of vanadium pentoxide and zinc oxide.[2] Since rubber mixings often contain barium and calcium sulphate or carbonate as pigment extenders or inert fillers, the use of vanadium pentoxide - zinc oxide mixture in conjunction with the combustion procedure offers the possibility of a rapid technique for the determination of total sulphur (*i.e.*, "organic" and "inorganic" combined) in such mixings.

As many of the newer synthetic polymers give rise, on heating, either to hydrochloric acid or to oxides of nitrogen, the determination of sulphur in mixings based on such polymers must be concluded with a specific reaction for sulphate. Belcher, Nutten and Stephen[3,4] have developed 4-amino-4′-chlorodiphenyl hydrochloride for use as a precipitant for sulphate ions, and have assessed its value in a general way. The sulphate of this organic base has the merit that it can be titrated with standard alkali, thus gaining considerably in time over the traditional gravimetric determination as barium sulphate.

Experience over several months has shown that the method gives a satisfactory degree of reproducibility and the level of accuracy lies within ± 1 per cent. of the correct figure.

Experimental and results

Apparatus—

The apparatus has been fully described and the procedure given in detail elsewhere.[2] The important dimensions of its principal parts, which have not been recorded previously, are as follows—

Combustion tube—The main tube is of translucent silica, 41 to 42 cm long and 16 mm bore. To this is fused a narrower tube of 7 mm bore in transparent silica of 17 cm length. This tube is then bent vertically downwards for 5 cm and terminates in a B10 cone for attachment to the absorber.

Absorption train—The absorption train consists of two vessels. The first is a modified Grote absorber,[5] without bead filling, of 40 ml capacity with a sintered disc of porosity 2 or 3.[6] The second absorption vessel is a pear-shaped 25-ml flask with inclined side-arm and is fitted with a gas-delivery tube extending to within 1 mm of the bottom.

ANALYSIS OF ALKALI AND ALKALINE-EARTH SULPHATES—

The technique has been used to verify the sulphur content of a variety of inorganic sulphur compounds used in the rubber industry. The materials chosen, although differing widely in function, are alike in that they do not decompose readily at temperatures below 1000° C. Other inorganic sulphur compounds are, of course, used in rubber compounding or are formed during vulcanisation, but their thermal decomposition in combustion techniques generally presents no difficulty.

In Table I the results of these determinations are collected together and the effect on the yield of sulphur from calcium sulphate of varying the composition of the catalyst is also shown. The weight of catalyst in all tests was 1 g and the combustion temperature was maintained at 1000° C for 30 minutes with sample weights in the range 10 to 15 mg.

TABLE I

SULPHUR CONTENT OF INORGANIC COMPOUNDS

Sample	Yield of sulphur, % of theoretical	Catalyst
Calcium sulphate	77·7, 77·0	V_2O_5
Calcium sulphate	97·7	V_2O_5 + Na_2O_2
Calcium sulphate	99·6, 100·0, 99·8	V_2O_5 (4 parts) + ZnO (1 part)
Barytes (natural)	88·8, 89·0, 89·0	
Barytes (a "purified" grade) ..	99·3, 99·5, 99·2	
Blanc fixé	88·3, 88·5	
Lithopone	97·1, 97·7	
Potassium sulphate	98·95, 98·92, 98·98	
Sodium sulphate	98·95, 99·06, 99·10	

Table I shows that when vanadium pentoxide is used alone as catalyst, decomposition of calcium sulphate is not complete in 30 minutes, but a mixed catalyst, containing both zinc oxide and vanadium pentoxide, induces a much faster decomposition rate, and liberation of the theoretical amount of sulphur is accomplished in a reasonable combustion time. The results from other sulphates, given in Table I, show a somewhat lower yield of sulphur than would be expected by calculation for the pure substance, but only commercial grades of these materials were examined. There is no reason to suppose that all the sulphur in these materials is not completely expelled, and compounded rubbers containing them as compounding ingredients can be expected to give a quantitative yield of their total sulphur content when subjected to the stated conditions of heating.

EFFECT OF CALCIUM CARBONATE—

Since the decomposition temperature of calcium sulphate is well above that of calcium carbonate, the former compound will tend to be formed preferentially when the carbonate is heated in presence of sulphur dioxide and trioxide. Consequently, when sulphur in a vulcanised rubber containing whiting is determined by the conventional combustion procedure, low results are invariably obtained. If, however, the silica[1] is replaced by a mixed vanadium pentoxide - zinc oxide catalyst, quantitative liberation of all forms of sulphur is achieved. Thus, in a laboratory prepared mixture containing 5·6 per cent. of sulphur and 10 per cent. of calcium carbonate, the sulphur recovered was 5·64 per cent. (mean of 4 results; range 5·62 to 5·67 per cent.).

ANALYSES OF SYNTHETIC RUBBER VULCANISATES AND ORGANIC COMPOUNDS—

Belcher and his co-workers[3,4] have suggested the use of 4-amino-4′-chlorodiphenyl hydrochloride as a reagent for sulphate, claiming that it forms an insoluble salt unaffected by nitrate, chloride and zinc. In general, these findings with respect to interference have been confirmed, but in this laboratory low and erratic recoveries of sulphur were obtained. The discrepancy was traced to the slight solubility of the freshly precipitated 4-amino-4′-chlorodiphenyl sulphate in the water used for washing the precipitate. Working with standard sulphate solutions, however, it was found that completely quantitative recovery of sulphate could be obtained when the wash water was replaced by a saturated solution of the amine sulphate (see Table II). This procedure was followed throughout subsequent work. The very small amount of wash liquid remaining in the filter-paper may be compensated for in the blank determination.

Reagent blanks are normally carried out by omitting the sample and proceeding as in an actual determination. The use of glass-fibre filter-mat in place of paper pulp reduces the reagent blank values to a negligible figure. For the quantitative retention of the amine sulphate, it was found essential to place in the Gooch crucible first a thin layer of pulp filter and, after tamping down gently the surface of the pad, a circle of glass-fibre filter-paper. The titration of the amine sulphate in the presence of glass-fibre filter-paper is improved to give a sharper end-point.

Table II summarises the results obtained by precipitating the amine sulphate in the presence of substances that are often present during the analysis of a synthetic rubber Aliquots of potassium sulphate solution were used to provide standard amounts of sulphate in the test solutions.

TABLE II

RECOVERY OF SULPHATE IN PRESENCE OF NITRATE, HYDROGEN PEROXIDE AND ZINC CHLORIDE

Interfering substance			Sulphur present, mg	Sulphur found, mg
Hydrogen peroxide, ml	Potassium nitrate, mg	Zinc chloride, mg		
—	—	—	0·500	0·500, 0·501, 0·499
—	—	—	2·00	2·00, 1·99, 1·99
20	—	—	2·10	2·10, 2·09, 2·10
20	—	0·35	0·500	0·500, 0·498, 0·499
—	8	—	2·10	2·09, 2·10, 2·10
20	8	—	2·10	2·09, 2·09, 2·10

TABLE III

SULPHUR IN SYNTHETIC RUBBER COMPOUNDS

Elastomer	Polymer type	Nitrogen, %	Chlorine, %	Sulphur found, %	Sulphur present, %
Hycar	Polyacrylonitrile - polybutadiene	8·35	—	2·42, 2·47	2·41
Polysar	Polyacrylonitrile - polybutadiene	6·86	—	2·56, 2·51	2·50
Neoprene	Polychloroprene	—	25·38	0·61, 0·59	0·63
Krylene	Polystyrene - polybutadiene	—	—	1·37, 1·37	1·38
Krylene - Hypalon mixture	Polystyrene - polybutadiene + chlorosulphonated polyethylene	—	8·64	0·67, 0·66	0·66
Hypalon - natural rubber mixture	Chlorosulphonated polyethylene + polyisoprene	—	8·64	0·66, 0·62, 0·62, 0·61	0·66
Hypalon - Hycar mixture	—	3·51	8·64	1·00, 1·02	1·06
Butyl - natural rubber mixture	Poly*iso*butene + polyisoprene	—	—	1·86, 1·85	1·88
Hypalon - Butyl mixture		—	8·13	0·96, 0·97	0·99

TABLE IV

SULPHUR CONTENT OF PURE ORGANIC COMPOUNDS

Compound	Sulphur found, %	Theoretical sulphur content, %
Sulphonal	28·03, 28·17, 27·98, 28·05, 27·98, 28·08	28·09
Sulphonal + dinitrobenzene ..	27·97, 27·98, 28·03, 27·99, 27·98	28·09
Sulphonal + iodobenzoic acid ..	27·94, 27·99	23·09
S-Benzylthiouronium chloride ..	15·86, 15·81, 15·83, 15·86, 15·81, 15·80	15·82
Phenylthiourea	21·03, 21·01, 21·05, 21·05, 21·05	21·07
2-Mercaptobenzothiazole ..	38·31, 38·32, 38·31, 38·33, 38·32	38·34
Iodobenzothiazole	12·23, 12·21, 12·24, 12·23, 12·21, 12·23	12·26

The results given in Table III relate to the determination of the synthetic rubber compounds. They show that a satisfactory degree of concordance is achieved between the calculated weight of added sulphur and the amount disclosed by analysis.

As a final evaluation, the method was applied to the determination of sulphur in a number of organic compounds. The results are given in Table IV.

The author thanks the Dunlop Rubber Company for permission to publish this paper.

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A Spectrophotometric Method for the Determination of Sub-micro Quantities of Sulphur with 4-Amino-4′-chlorodiphenyl

By A. S. Jones and D. S. Letham

(*Presented at the meeting of the Midlands Section on Wednesday, May 25th*, 1955)

4-Amino-4′-chlorodiphenyl has been used to determine 30 to 120-μg quantities of sulphate. The amount of reagent combining with the sulphate was calculated by difference from measurement of the amount remaining in solution after precipitation of the 4-amino-4′-chlorodiphenyl sulphate. The concentration of 4-amino-4′-chlorodiphenyl was determined by ultra-violet spectrophotometry. Interfering phosphate could be removed by precipitation as magnesium ammonium phosphate. By use of a suitable digestion procedure the method was used to determine 20 to 50 μg of sulphur in organic compounds.

During work on the determination of the nucleotide sequence of deoxypentosenucleic acids, with the use of thioglycollic acid and then degradation with alkali,[1] it was necessary to determine 20 to 50-μg quantities of sulphur. Since the reagent used by Belcher, Nutten and Stephen[2] (4-amino-4′-chlorodiphenyl) appeared to be promising, a method involving its use for the determination of these quantities has been developed. A brief note of this method has already been published.[3]

4-Amino-4′-chlorodiphenyl showed a strong absorption in the ultra-violet region, and so it was decided to determine the concentration of the reagent spectrophotometrically, thus avoiding the titration of small quantities. The optical properties of the reagent were satisfactory, the material being homogeneous when fractionated with sulphate and obeying Beer's law at the required concentrations (2 to 11 μg per ml). In order to obviate errors due to washing the precipitate, a difference method was used, in which the amount of reagent remaining in solution after precipitation of the sulphate was determined.

To determine inorganic sulphate, an excess (at least 100 per cent.) of 4-amino-4′-chlorodiphenyl in 0·1 *N* hydrochloric acid was added to the sulphate solution at pH 2 to 7, and the precipitate was removed by centrifugation. The sulphate precipitate tended to adhere to the surface of the liquid after centrifuging, but this difficulty was overcome by adding a trace of Cetavlon (cetyltrimethylammonium bromide), when the precipitate separated completely to the bottom of the tubes. Phosphate, which interfered with the sulphate determination, could be removed by precipitation as magnesium ammonium phosphate. This precipitate could be used for the determination of phosphorus. In removing phosphate

care was taken to add no more than 9 mg of ammonium chloride and 4·5 mg of magnesium chloride dihydrate per millilitre of final solution in order to avoid salting out 4-amino-4′-chlorodiphenyl hydrochloride. At these concentrations magnesium did not interfere with the determination of sulphate.

For the conversion of "organic sulphur" to sulphate a digestion method was used in order to avoid the production of solutions of high ionic concentration, which would salt out the 4-amino-4′-chlorodiphenyl reagent. Of several methods tried, the best for nucleic acid fragments (which contained sodium, phosphorus and sulphur) was digestion with concentrated nitric acid and a small amount of sodium chloride in a sealed tube at 280° for 3 hours.

Experimental

Optical properties of 4-amino-4′-chlorodiphenyl—

The reagent was recrystallised from light petroleum, boiling range 80° to 100° C. Colourless plates were obtained, m.p. 132° C (uncorrected). Further recrystallisation did not alter the melting point or change the ultra-violet absorption spectrum. The absorption spectrum of 4-amino-4′-chlorodiphenyl in 0·1 *N* hydrochloric acid is shown in Fig. 1; $\lambda_{max} = 254$ mμ

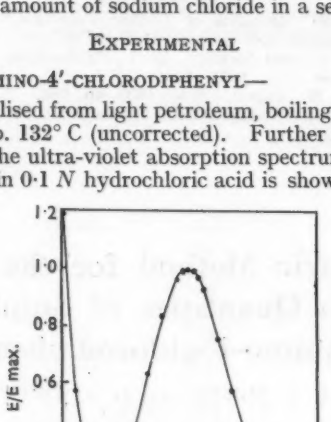

Fig. 1. Absorption spectrum of 4-amino-4′-chlorodiphenyl in 0·1 *N* hydrochloric acid

and $\epsilon_{254} = 22{,}090$. These constants were determined by dissolving 4-amino-4′-chlorodiphenyl (5 to 7 mg dried at 80° C and 15 mm pressure) in 0·1 *N* hydrochloric acid (500 ml).

Method

Procedure for determining sulphate—

The following method may be used to determine 30 to 120 μg of sulphate.

Add a trace of Cetavlon and 0·50 ml of 4-amino-4′-chlorodiphenyl reagent (a 0·19 per cent. solution of the base in 0·1 *N* hydrochloric acid) to (*i*) the sulphate solution, and (*ii*) an

Table I

Typical recovery experiment

	Volume of sulphate solution* taken, ml	Volume of water added, ml	Volume of reagent, ml	Optical density at 254 mμ†	Difference between test and blank, (*ii*–*i*)	Sulphate found, μg	Sulphate calculated, μg
(*ii*)	0·000	0·500	0·500	1·177	—	—	—
(*i*)	0·200	0·300	0·500	0·528	0·649	117	116·1
	0·150	0·350	0·500	0·698	0·479	86·5	87·0
	0·120	0·380	0·500	0·792	0·385	69·5	69·6
	0·100	0·400	0·500	0·855	0·322	58·1	58·05
	0·050	0·450	0·500	1·015	0·162	29·2	29·0

* 0·5805 mg of sulphate per ml. † Mean of two readings.

equal volume of distilled water. After mixing, keep the solutions for 2 hours and then centrifuge them. Remove 0·3-ml aliquots of the supernatant liquids with a micrometer syringe and dilute them to 25 ml with 0·1 *N* hydrochloric acid. Read the optical densities at 254 mμ in 1-cm cells. From the difference of the optical densities in (*i*) and (*ii*), and the specific extinction coefficient of 4-amino-4′-chlorodiphenyl, calculate the amount of reagent precipitated and hence determine the sulphate content.

Results—The results of a typical recovery experiment are recorded in Table I.

PROCEDURE FOR DETERMINING SULPHATE IN THE PRESENCE OF PHOSPHATE—

To the sulphate solutions containing 0·5 ml of phosphate solution (about 400 μg per ml) in centrifuge tubes, add 0·04 ml of magnesium chloride - ammonium chloride reagent (100 mg of $MgCl_2.6H_2O$ per ml; 200 mg of NH_4Cl per ml), 0·02 ml of aqueous ammonia, sp.gr. 0·880, and a trace of Cetavlon in that order. Mix the solutions, leave them overnight and then centrifuge them. Transfer aliquots of the supernatant liquids to centrifuge tubes, dilute with 0·3 ml of water and acidify with hydrochloric acid to pH 2 to 3. Sulphate is determined by using the 4-amino-4′-chlorodiphenyl reagent as before.

Results—Typical results are as follows:—

Sulphate recovered, μg ..	88·2, 87·6, 87·4	71·0, 70·1	59·0, 58·6
Mean value, μg	87·7	70·5	58·8
Theoretical value, μg	87·0	69·5	58·1

PROCEDURE FOR DETERMINING SULPHUR IN ORGANIC COMPOUNDS—

Oxidise samples of the substance (containing 20 to 50 μg of sulphur) in sealed tubes at 280° C with concentrated nitric acid for 3 hours as follows[4]—

Digestions (*i*) and (*ii*); substance, 0·6 mg of sodium chloride and 0·2 ml of concentrated nitric acid.

Digestion (*iii*); 0·6 mg of sodium chloride and 0·2 ml of concentrated nitric acid.

Use digestion (*i*) for the determination of sulphur and digestion (*iii*) for a sulphur blank on the reagents. Use digestion (*ii*) as an "ultra-violet" blank to determine whether ultra-violet-absorbing material is produced during the digestion procedure. Concentrate the digests to dryness by evaporating in a current of warm air. Dissolve the residues from (*i*) and (*iii*) in distilled water and determine the sulphate content. Dissolve the residue from (*ii*) in 1 ml of distilled water and dilute a 0·3-ml aliquot to 25 ml with 0·1 *N* hydrochloric acid and measure the optical density at 254 mμ. With the compounds used in this work (mercaptals of thioglycollic acid) these "ultra-violet" blanks were always negligible.

The method was used to analyse thioglycollated calf-thymus deoxyribonucleic acid, which had been analysed by a micro adaptation of the titrimetric method of Belcher *et al.*[2]

Found by titrimetric method, 3·94 atoms of sulphur per 4 atoms of phosphorus.

Found by spectrophotometric method, 3·89 atoms of sulphur per 4 atoms of phosphorus.

Calculated (from purine content of intact deoxyribonucleic acid), 3·88 atoms of sulphur per 4 atoms of phosphorus.

The titrimetric method was found to give good results (see Table II) with simple mercaptals and with mercaptals in the presence of deoxyribonucleic acid, the same method being used for the conversion of "organic sulphur" to sulphate and for the removal of phosphate as described above.

TABLE II

DETERMINATION OF "ORGANIC SULPHUR"

Compound	Sulphur found, %	Theoretical value, %
Glucose diethylmercaptal	22·2, 22·4, 22·2	22·37
Benzaldehyde di(carboxymethyl)mercaptal	23·3, 23·3	23·53
Glucose diethylmercaptal + deoxyribonucleic acid ..	22·2, 22·2, 22·4	22·37

DISCUSSION OF RESULTS

By using the procedure outlined above, recoveries of sulphate were within 1 per cent. of theoretical and within 2 per cent. when removal of phosphate was necessary. Recoveries of sulphur from organic compounds (mercaptals of thioglycollic acid) were within 2 per cent. of theoretical. The accuracy is, therefore, at least as good as that of the best existing sub-micro method (Johnson and Nishita[5]). The present method, however, is simpler than that

due to Johnson and Nishita in that no complicated distillation apparatus is required and it may, therefore, be used for the simultaneous analysis of a number of samples. Another advantage is that considerable quantities of nitrate can be tolerated.

The authors thank Professor M. Stacey, F.R.S., for his interest in this work, Dr. R. Belcher for many helpful discussions and the University of Birmingham for a special grant from the Research Fund.

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The Analysis of Mixtures of Surface-active Quaternary Ammonium Compounds and Polyethylene Oxide Type of Non-ionic Surface-active Agents

BY A. BARBER, C. C. T. CHINNICK AND P. A. LINCOLN

The interference of polyethylene oxide "non-ionic" agents with the bromophenol blue method of determining quaternary ammonium compounds has been described and a method for overcoming this interference has been proposed.

The determination of polyethylene oxide "non-ionic" agents by precipitation with a heteropoly acid has been improved and has been shown to be applicable to three different "non-ionic" agents.

The removal of quaternary ammonium cations, which interfere with the determination of polyethylene oxide "non-ionic" agents, has been shown to be possible with an ion-exchange resin.

Two examples of the application of the methods to typical commercial products are described.

THE use of mixtures of surface-active quaternary ammonium compounds and polyethylene oxide type non-ionic surface-active agents ("non-ionics") in such products as detergent sterilisers and pharmaceutical or cosmetic emulsions is now quite common. There have not, however, been reported any methods for the quantitative determination of quaternary ammonium compounds and "non-ionics" in the presence of one another.

The bromophenol blue method of Barr, Oliver and Stubbings[1] can be used to assay the quaternary ammonium compound, but it has been found that conditions must be standardised to give accurate results in the presence of "non-ionic" agents.

For the determination of polyethylene oxide "non-ionics" the method of Oliver and Preston[2] has been improved by replacing phosphomolybdic acid with phosphotungstic acid. The determination of "non-ionics" in the presence of quaternary ammonium compounds is complicated by the fact that the latter form insoluble phosphotungstates.[3] Contrary to the observations of Heatley and Page,[4] we found that quaternary ammonium cations can be removed quantitatively from solution by a cation-exchange resin, leaving the "non-ionic" in solution to be assayed by the prescribed method. It has also been reported by Green, Harker and Howitt[5] that an anion-exchange resin will absorb cetyltrimethylammonium bromide from aqueous solution, but this method was not tested in the present investigation.

Experimental

Materials used—

Quaternary ammonium compounds—The quaternary ammonium compounds used were chemically pure compounds (these compounds are available, some at present only in limited quantities, from Milton Industrial Chemicals (London) Ltd. and by arrangement through most laboratory chemical supply houses) made from the appropriate fractionally distilled alkyl halides and amines, followed by recrystallisation from a suitable solvent. Solutions (0·005 *N*) were prepared by dissolving the required amount of material in water and standardising the solution with 0·01 *N* silver nitrate according to the method of Dubois.[6]

"Anionic" reagent—The "anionic" reagent used in the bromophenol blue method was a 0·01 *N* solution of sodium dioctylsulphosuccinate (this is available in a pure form as "Manoxol OT 100%"; the purity can be checked by determination of saponification value). This "anionic" was chosen because it is readily available in a pure form and has a high molecular weight. It has been observed that some less pure commercial "anionic" detergents that have been used in bromophenol blue titrations do not give end-points as sharp as those obtained when sodium dioctylsulphosuccinate is used. It has been reported elsewhere[3] that "anionic" materials of low molecular weight do not react stoicheiometrically with quaternary ammonium compounds under the conditions of the titration.

"Non-ionic" agents—Three compounds were chosen as representing different types of polyethylene oxide non-ionic surface-active agents—

"Non-ionic A": octylphenol condensed with ethylene oxide to give a compound with an average molecular weight of 530 (Lissapol NX, supplied by Imperial Chemical Industries Ltd., was used as representative of this type of material).

"Non-ionic B": commercial cetyl alcohol condensed with ethylene oxide to give a compound with an average molecular weight of 1030 (Lubrol W, supplied by Imperial Chemical Industries Ltd., was used as representative of this type of material).

"Non-ionic C": a polyethylene glycol of average molecular weight 400 esterified with lauric acid, the resulting compound having an average molecular weight of 580 (Nonex 139B, supplied by Gemec Chemical Co. Ltd., was used as representative of this type of material).

Solutions (0·1 *N*) of the above materials were made by dissolving the required amount in water and making up to 1 litre.

Reagents—

Bromophenol blue—A 0·1 per cent. solution in ethanol.
Sodium chloride—A 20 per cent. solution in water.
Sodium carbonate—A 10 per cent. solution in water.
Hydrochloric acid—A 10 per cent. solution in water.
Barium chloride—A 10 per cent. solution in water.
Phosphomolybdic acid—A 10 per cent. solution of $P_2O_5.12MoO_3.xH_2O$ in water.
Phosphotungstic acid—A 10 per cent. solution of $P_2O_5.12WO_3.xH_2O$ in water.

Determination of quaternary ammonium compounds—

The bromophenol blue method of Barr, Oliver and Stubbings[1] for the determination of "anionic" detergents by using a quaternary ammonium compound as titrant has been shown to be applicable to the analysis of a wide range of quaternary ammonium compounds.[3] An alkaline solution of "anionic" detergent in the presence of bromophenol blue and ethylene dichloride is titrated with a standard quaternary ammonium compound solution. The end-point is indicated by the change colourless - green - deep blue in the ethylene dichloride layer. For determination of an unknown quaternary compound a portion is added to the "anionic" and the excess of "anionic" is titrated with the standard quaternary ammonium compound solution.

With pure quaternary ammonium compounds and "anionics" the change from colourless - green - deep blue requires about 0·3 ml of titrant, and the end-point is easily reproduced to 0·1 ml. In the presence of "non-ionics" the green intermediate stage can persist during the addition of 2 to 3 ml of titrant. An accurate end-point can be obtained by first carrying out a blank titration with no "non-ionic" present and using the blue ethylene dichloride layer of this as a match for the titration of the sample containing some "non-ionic."

In addition, whereas it has been found that with pure quaternary ammonium compounds and "anionic" detergents up to 10 g or so of inorganic salts did not affect the end-point, in the presence of those "non-ionics" examined the truest end-point was obtained only if the sodium chloride content was not less than 1·25 and not more than 2·5 g.

Results—The results illustrating these points are given in Tables I, II and III; the results in Table III are plotted in Fig. 1. The method used to obtain these results was as follows.

Procedure—By pipette put 10 ml of sodium dioctylsulphosuccinate solution into a 100-ml stoppered measuring cylinder. Add 20 ml of ethylene dichloride, the required volume of

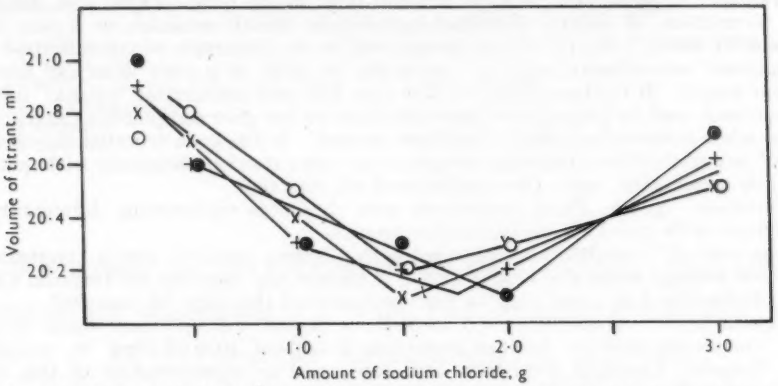

Fig. 1. Graphical representation of results in Table III. ×, 1 mole of non-ionic agent A; +, 2 moles of non-ionic agent A; O, 1 mole of non-ionic agent B; ●, 2 moles of non-ionic agent B

"non-ionic" and sodium chloride solutions, 1 ml of bromophenol blue and a few drops of sodium carbonate to make alkaline, and make up to 80 ml with water. Titrate with quaternary ammonium compound solution. The end-point is noted when the deep blue colour of the ethylene dichloride layer matches that of a titration carried out in the absence of a "non-ionic" agent.

TABLE I

EFFECT OF VARIOUS AMOUNTS OF "NON-IONIC A" ON THE TITRATION OF SODIUM DIOCTYLSULPHOSUCCINATE WITH CETYLTRIMETHYLAMMONIUM CHLORIDE SOLUTION

2·0 g of sodium chloride in each sample

Ratio of moles of "non-ionic A" to moles of sodium dioctylsulphosuccinate	0	1	2	5	10
Titre to first appearance of green colour in ethylene dichloride, ml	19·8	19·0	18·7	17·8	16·8
Titre to matching deep blue colour in ethylene dichloride, ml	20·1	20·1	20·2	20·1 ± 0·1	20·0 ± 0·2

TABLE II

EFFECT OF "NON-IONIC A" ON THE TITRATION OF SODIUM DIOCTYLSULPHOSUCCINATE WITH CETYLTRIMETHYLAMMONIUM CHLORIDE, LAURYLTRIMETHYLAMMONIUM CHLORIDE AND CETYLPYRIDINIUM CHLORIDE

2·0 g of sodium chloride in each sample

Quaternary ammonium compound	Cetyltrimethyl-ammonium chloride		Lauryltrimethyl-ammonium chloride		Cetylpyridinum chloride	
Ratio of moles of "non-ionic A" to moles of sodium dioctylsulphosuccinate	0	5	0	5	0	5
Titre to first appearance of green in ethylene dichloride, ml	19·8	17·8	19·7	17·6	19·9	17·8
Titre to matching deep blue colour in ethylene dichloride, ml	20·1	20·1 ± 0·1	20·0	19·8 ± 0·1	20·2	20·3 ± 0·1

TABLE III

EFFECT OF VARIOUS QUANTITIES OF SODIUM CHLORIDE ON THE TITRATION OF SODIUM DIOCTYLSULPHOSUCCINATE WITH CETYLPYRIDINIUM CHLORIDE SOLUTION, WITH AND WITHOUT "NON-IONIC A" OR "NON-IONIC B"

	Volume of cetylpyridinium chloride solution required with				
		"non-ionic A" present with dioctylsulphosuccinate in		"non-ionic B" present with dioctylsulphosuccinate in	
Sodium chloride present, g	no "non-ionic" present, ml	equimolar proportions, ml	molar ratio of 2 to 1, ml	equimolar proportions, ml	molar ratio of 2 to 1, ml
0·25	20·2	20·8	20·9	20·7	21·0
0·5	20·2	20·7	20·6	20·8	20·6
1·0	20·2	20·4	20·3	20·5	20·3
1·5	20·2	20·1	20·2	20·2	20·3
2·0	20·2	20·3	20·2	20·3	20·1
3·0	20·2	20·5	20·6	20·5	20·7

DETERMINATION OF NON-IONIC SURFACE-ACTIVE AGENTS—

For the assay of non-ionic surface-active agents the most convenient method available is the barium chloride - phosphomolybdic acid precipitation method of Oliver and Preston.[2] This is adapted from the method used by Schaffer and Critchfield[7] for the analysis of a commercial range of polyethylene glycols. It consists in precipitating a complex of the "non-ionic" agent with barium chloride and a heteropoly acid and either weighing the complex dried at 105° C or igniting the precipitate and weighing the metal oxide residue. This must be related to the original amount of "non-ionic" agent by means of an empirical factor or calibration curve determined by precipitating known amounts of "non-ionic" agent. It is therefore necessary to know the nature of the "non-ionic" agent present in an unknown sample.

It was found in early tests on the method with "non-ionic A" that phosphotungstic acid gave better results than phosphomolybdic acid. Silicotungstic acid was also tried, but this gave gummy precipitates, and errors of 15 per cent. were possible. Phosphotungstic acid not only gave more accurate results than phosphomolybdic acid, but it was also found that by using the former acid the precipitate could be filtered off after 2 to 3 hours, whereas with phosphomolybdic acid it was necessary to leave overnight before filtering.

Procedure—To the "non-ionic" sample in 200 ml of water add 10 ml of hydrochloric acid and 10 ml of barium chloride solution, and raise the solution to boiling. Then add 10 ml of heteropoly acid solution to the solution and continue boiling for 1 to 2 minutes to coagulate the precipitate. Set the mixture aside overnight or for 2 to 3 hours, depending on which acid is used, collect the precipitate on a weighed No. 3 sintered-glass crucible, wash it with about 100 ml of water and dry it to constant weight at 105° C.

Results—Some results obtained by this method are shown in Tables IV, V, VI and VII,

TABLE IV

RESULTS OBTAINED WITH "NON-IONIC A" AND PHOSPHOMOLYBDIC ACID AFTER THE MIXTURE HAS BEEN SET ASIDE OVERNIGHT BEFORE FILTERING

Weight of "non-ionic A," mg	Weight of precipitate, mg			Average weight, mg	Average deviation, %	Ratio of weight of precipitate to weight of "non-ionic A"
200	618·9	600·1	599·7	606·2	1·4	3·031
100	312·2	302·2	314·3	309·6	1·6	3·096
50	146·8	146·6	158·8	150·7	3·6	3·014
25	86·7	73·4	80·6	80·2	5·7	3·208

from which it can be seen that with the three "non-ionic" agents tested excellent results were obtained with phosphotungstic acid. If a calibration graph is constructed by plotting the weight of precipitate against weight of "non-ionic" agent, a straight line results in all three cases (see Fig. 2).

The interference caused by quaternary ammonium compounds in the use of the above method is due to the formation of insoluble quaternary ammonium phosphotungstates of

TABLE V

RESULTS OBTAINED WITH "NON-IONIC A" AND PHOSPHOTUNGSTIC ACID AFTER THE MIXTURE HAS BEEN SET ASIDE FOR 2 TO 3 HOURS BEFORE FILTERING

Weight of "non-ionic A," mg	Weight of precipitate, mg			Average weight, mg	Average deviation, %	Ratio of weight of precipitate to weight of "non-ionic A"
200	724·0	723·1	724·6	723·9	0·1	3·619
100	369·0	368·6	367·3	368·3	0·5	3·683
50	190·6	191·5	193·0	191·7	0·4	3·834
25	103·8	104·0	99·4	102·4	2·0	4·096
12·5	47·9	51·2	50·2	49·8	3·0	3·984

TABLE VI

RESULTS OBTAINED WITH "NON-IONIC B" AND PHOSPHOTUNGSTIC ACID AFTER THE MIXTURE HAS BEEN SET ASIDE FOR 2 TO 3 HOURS BEFORE FILTERING

Weight of "non-ionic B," mg	Weight of precipitate, mg			Average weight, mg	Average deviation, %	Ratio of weight of precipitate to weight of "non-ionic B"
200	937·1	931·2	932·8	933·4	0·2	4·667
100	466·1	467·6	466·6	467·7	0·2	4·677
50	232·6	231·4	230·2	231·4	0·4	4·628
25	114·7	116·4	115·1	115·4	0·6	4·616

TABLE VII

RESULTS OBTAINED WITH "NON-IONIC C" AND PHOSPHOTUNGSTIC ACID AFTER THE MIXTURE HAS BEEN SET ASIDE FOR 2 TO 3 HOURS BEFORE FILTERING

Weight of "non-ionic C," mg	Weight of precipitate, mg			Average weight, mg	Average deviation, %	Ratio of weight of precipitate to weight of "non-ionic C"
200	888·5	884·4	878·9	882·9	0·9	4·414
100	444·1	448·1	441·8	444·7	0·5	4·447
50	226·8	226·1	223·0	225·3	0·2	4·506
25	114·7	117·3	122·3	118·1	0·7	4·724

the formula $Q_3PO_4.12WO_3$. The precipitation of these insoluble phosphotungstates has, in fact, been shown to be an excellent method for the assay of quaternary ammonium compounds.[3]

The quaternary ammonium cations can, however, be removed easily from "cationic - non-ionic" mixtures by means of a cation-exchange resin. It was found that leaving 2 to 3 ml of Zeo-Karb 225(H) (a sulphonated polystyrene cation-exchange resin supplied by The Permutit Co. Ltd.) in contact with 50 ml of 0·005 *N* quaternary ammonium compound solution for ½ to 1 hour with occasional shaking resulted in quantitative removal of the

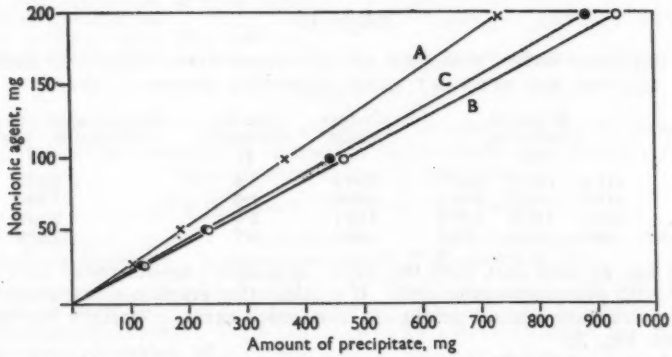

Fig. 2. Graphical representation of results: A, results from Table V; B, results from Table VI; C, results from Table VII

quaternary ammonium cations. In the presence of "non-ionic" agents the quaternary ammonium cations were still quantitatively removed. The following quaternary ammonium compounds were tested for absorption: cetyltrimethylammonium bromide, myristylpyridinium chloride, cetyldimethylbenzylammonium chloride, cetylbenzylmorpholinium chloride, laurylmethyldiethanolammonium chloride and *p-tert.*-octylphenoxyethoxyethyldimethylbenzylammonium chloride (Hyamine 1622). The quaternary ammonium compounds were all absorbed by the resin in the presence of 100 mg of "non-ionic A." Cetyltrimethylammonium ions were still absorbed in the presence of 500 mg of "non-ionic A," "non-ionic B" or "non-ionic C."

Some quantitative tests were carried out by precipitating known mixtures of quaternary ammonium compound and "non-ionic A" before and after treatment with Zeo-Karb 225 (H), *i.e.*, precipitating the "non-ionic" - phosphotungstate complex together with the quaternary ammonium phosphotungstate and then the "non-ionic" - phosphotungstate complex after removing the quaternary ammonium cations.

Mixed solutions, 0·005 *N* with respect to quaternary ammonium compound and containing 2·000 g of "non-ionic A" per litre, were prepared.

Procedure for precipitating the quaternary ammonium compound and "non-ionic"—By pipette put 50 ml of mixture in a 400-ml beaker and make up to 200 ml with water. Add 10 ml of hydrochloric acid and 10 ml of barium chloride solution, and bring to the boil. Add 10 ml of phosphotungstic acid solution, and boil for 1 to 2 minutes to coagulate the precipitate. Cool the solution and set it aside for 2 to 3 hours; collect the precipitate on a No. 3 sintered-glass crucible, wash it with about 100 ml of water in 15 to 20 ml aliquots, and then dry it at 105° C.

Procedure for precipitating "non-ionic" agent only—By pipette put 50 ml of solution in a 100-ml conical flask, add 5 ml of Zeo-Karb 225(H) and leave for ½ to 1 hour with occasional shaking. Decant off the solution from the resin and wash the resin with six 25-ml portions of water, adding the washings to the solution. Precipitate the "non-ionic" - phosphotungstate complex as before.

Results—Some results obtained by this method are shown in Table VIII.

TABLE VIII

DETERMINATION OF SOME QUATERNARY AMMONIUM COMPOUNDS AS PHOSPHOTUNGSTATES IN THE PRESENCE OF "NON-IONIC A" AND "NON-IONIC B"

Quaternary ammonium compound	"Non-ionic A," mg	Weight of precipitate of total quaternary ammonium compounds plus "non-ionic A" precipitate: Weight of duplicates, mg	Average (A), mg	Weight of "non-ionic" precipitate: Weight of duplicates, mg	Average (B), mg	Weight of quaternary precipitate calculated, mg	Weight of quaternary precipitate by difference (A–B), mg
Myristylpyridinium chloride	100	670·1 678·8	674·5	364·5 369·1	366·8	309·0	307·7
Cetyltrimethylammonium bromide	100	678·2 691·5	684·4	368·5 360·4	364·4	311·0	320·4
Decyltrimethylammonium bromide	100	650·6 656·3	653·4	369·5 377·4	373·4	290·0	280·0
p-tert.-Octylphenoxy-ethoxyethyldimethyl-benzylammonium chloride	100	710·5 714·5	712·5	364·8 371·2	368·0	343·0	344·5

TYPICAL RESULTS

It can be seen that the weight of "non-ionic A" - phosphotungstate complex precipitate obtained after removal of the quaternary ammonium cations by means of Zeo-Karb 225(H) agrees very well with the figure given in Table V for 100-mg amounts of "non-ionic A."

SOME TYPICAL DETERMINATIONS

The precise method of procedure with a sample containing both cationic and non-ionic surface-active agents will clearly depend on the nature and composition of the sample.

Thus, certain detergent - sanitisers may be shown by the standard techniques of qualitative analysis to consist solely of mixtures of quaternary ammonium compounds and "non-ionic" materials: with such mixtures the methods described previously are applied directly without modification, although an initial approximate determination may be required in order that a reasonable sample size may be taken for the analysis.

With other materials, normal analytical procedures will result in the isolation of an inorganic and an organic fraction, the former presenting no abnormal problem and the latter being dealt with as described above. With some such samples, particularly when the method is applied to quality control analysis of products of known composition, the modified bromophenol blue method may be employed for the quaternary ammonium compound, and the "non-ionic" calculated by difference. This may also be applied to aqueous solutions of the detergent - sanitiser class previously referred to, the water content being determined by the Dean and Stark method, the lengthier and direct gravimetric analysis for "non-ionic" detergent being thus avoided.

It is assumed that in general these methods will be applied to compounded mixtures of which the qualitative composition is known or can be determined by known techniques. Discussion of these techniques is beyond the scope of this paper, but some references to the literature on this subject are appended.[8,9,10,11,12] Familiarity with the commercial products available and their properties greatly assists the qualitative analysis of an "unknown," particularly since a result is frequently capable of more than one interpretation.

Two typical applications of the methods described to previously "difficult" analyses are as follows —

(*a*) A detergent - sanitiser powder was made up to contain 2 per cent. of cetyltrimethylammonium bromide and 3 per cent. of "non-ionic A," the remaining 95 per cent. consisting of inorganic builders (sodium carbonate, trisodium phosphate and sodium metasilicate) and water.

The quaternary ammonium was determined by the bromophenol blue method; 20 ml of 0·01 *N* sodium dioctylsulphosuccinate solution titrated with 0·005 *N* cetylpyridinium chloride solution gave a titre of 40·3 ml. To 20 ml of sodium dioctylsulphosuccinate were added 2·00 g of powder and the excess of reagent was titrated with 0·005 *N* cetylpyridinium chloride solution, to give a titre of 17·9 ml. Then—

$$\frac{(40{\cdot}3 - 17{\cdot}9) \times 364{\cdot}4 \times 0{\cdot}005 \times 100}{2{\cdot}000 \times 1000} = 2{\cdot}04 \text{ per cent. of cetyltrimethylammonium bromide.}$$

The "non-ionic" agent and total "non-ionic" - quaternary ammonium compound present were determined separately with phosphotungstic acid. A 25·0-g portion of powder was dissolved in 500 ml of water and extracted with *n*-butanol in a continuous extractor for 3 hours.[13] The *n*-butanol was removed under reduced pressure and the residue was taken up in water and made up to 250 ml in a calibrated flask. Two 25-ml samples of this solution were precipitated with phosphotungstic acid to give the total "non-ionic A" plus cetyltrimethylammonium complexes. Two further 25-ml samples were treated for 1 hour with 2 to 3 ml of Zeo-Karb 225(H), and the "non-ionic" agent remaining in the solution was precipitated with phosphotungstic acid.

The results of duplicate determination gave the total weight of precipitate as 432·7 and 441·4 mg (average, 437·0 mg) and of "non-ionic" precipitate as 266·4 and 274·2 mg (average, 270·3 mg); from the averages the weight of "non-ionic" was calculated to be 73·0 mg and the weight of cetyltrimethylammonium bromide was 48·8 mg.

From the calculated weights it can be deduced that the product contained 2·92 per cent. of "non-ionic A" and 1·95 per cent. of cetyltrimethylammonium bromide. The 1·95 per cent. for cetyltrimethylammonium bromide compares favourably with 2·04 per cent. as determined by the bromophenol blue method previously.

It should be noted that had the mixture employed in this example been an "unknown," the nature of the anion associated with the quaternary ammonium cation would require identification before the weight of the component present could be calculated; in this instance the presence of bromide ion would be found in both the *n*-butanol extract and in the aqueous organic phase, but since the bromide ion is foreign to inorganic constituents of detergents, it would clearly be attributable to the cationic surface-active agent present. Again, in the event that the exact nature of the quaternary ammonium

salt is unknown or has not been determined, the actual percentage and the mean molecular weight may be determined by the method of Chinnick and Lincoln.[3]

(*b*) A "non-ionic" emulsifying wax containing 10 per cent. of "non-ionic B" and 90 per cent. of cetostearyl alcohol was prepared. A 5·00-g portion of the wax was boiled with 500 ml of water and 20 ml of 10 per cent. hydrochloric acid, and 20 ml of 10 per cent. barium chloride solution were added. With stirring, 50 ml of 10 per cent. phosphotungstic acid solution were added, and boiling was continued for 10 minutes. The mixture was cooled and set aside for 2 to 3 hours, and then the mixed precipitate of "non-ionic B" complex and cetostearyl alcohol was collected on a weighed No. 3 sintered extraction thimble. The precipitate was sucked as dry as possible and then extracted in a Soxhlet extractor for 1 hour with light petroleum, boiling range 40° to 60°. The light petroleum dissoved the alcohol, the insoluble "non-ionic B" complex being left in the extraction thimble, which was reweighed after being dried at 105° C. The light petroleum solution of cetostearyl alcohol was evaporated on a steam-bath in a weighed beaker, the last traces of solvent being removed by blowing a current of air into the beaker. The beaker was reweighed to give the weight of cetostearyl alcohol.

The results of duplicate determinations gave the weight of "non-ionic B" complex as 2·4274 and 2·4108 g (average, 2·4191 g); the weight of "non-ionic B" calculated from the average weight was 0·5205 g. Duplicate determinations gave 4·4882 and 4·4073 g (average, 4·4477 g) for the weight of cetostearyl alcohol. From these figures it can be calculated that the wax contained 10·4 per cent. of "non-ionic B" and 88·9 per cent. of cetostearyl alcohol.

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Determination of Serum-protein Fractions by Zone Electrophoresis on Paper and Direct Reflection Photometry

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The use of direct reflection photometry for the evaluation of dyed serum-protein fractions, obtained by zone electrophoresis on paper, has been examined. The adsorption of certain dyes by protein precipitated on filter-paper was found to be approximately proportional to the protein concentration within certain limits. The reflection of light from the dyed protein fractions has been studied and a densitometer calibrated so that readings are proportional to protein concentration. By use of paper electrophoresis the protein in series of normal and pathological sera were examined; the results, and their reproducibilities, obtained by elution of the dye and by direct photometry have been compared. The validity of the results and those obtained by other electrophoretic methods are discussed.

THE uses and limitations of zone electrophoresis on paper for the analysis of serum proteins have recently been reviewed by Martin and Franglen.[1] Estimates of the relative concentrations of the major protein fractions may be obtained by staining the separated proteins and then either eluting the dye separately from each zone[2] or determining the dye by direct photometry. For the latter purpose photometers have been devised by Grassmann and Hannig,[3] Griffiths,[4] Crook, Harris, Hassan and Warren,[5] Schulz, Dryer and Holdcraft,[6] Discombe, Jones and Winstanley[7] and Laurence,[8] all of whom have measured the absorption of transmitted light, and by Rottger[9] and Latner, Molineux and Rose,[10] who determined the reduced intensity of light reflected from the stained zones.

Quantitative results are obtained after direct photometry by applying planimetry to areas under segments of the curve representing the distribution of protein fractions. The validity of these results depends on the demonstration of: (*a*) proportionality of dye concentration to concentration of protein in individual fractions, and (*b*) proportionality of the height of a point on the resulting curve to the concentration of the dye at the corresponding point on the stained electrophoresis strips. About the first of these requirements, Franglen and Martin,[11] Grassmann and Hannig[12] and Hardwicke[13] have reported that the amount of dye absorbed by protein is approximately proportional to the concentration of protein when the latter is small. About light absorption by dye absorbed to protein on paper, many workers have assumed that the optical density (transmission) of the dye is proportional to the concentration of the dye present, *i.e.*, it obeys Beer's law, and many photometers available commercially are calibrated accordingly. This assumption has received support from the findings of Grassmann and Hannig,[12] who, using naphthalene black, bromophenol blue and azocarmine as dyes, reported that the absorption of light by the dyed protein obeyed Beer's law, and from those of Latner *et al.*,[10] who, using naphthalene black as dye, came to a similar conclusion.

In contrast, however, Crook *et al.*,[5] using naphthalene black and azocarmine as dyes, and Hardwicke,[13] using bromophenol blue, have reported that the optical density is not proportional to the concentration of the dye present, and this is in agreement with the findings of Price, Hudson and Ashman,[14] who reported that various other substances when present on filter-paper fail to absorb transmitted light in accordance with Beer's law. It seems likely that these opposing conclusions are largely due to the use of photometers differing in their optical systems, light sources and filters and, possibly, also to the use of different methods of fixing and staining the protein fractions.

Using a photometer measuring reflected light, Latner *et al.*[10] reported that, with naphthalene black as dye, the reading obtained was proportional to the protein concentration. In view of this, and because this type of photometer does not require the paper to be rendered translucent, the use of an instrument of this type, as a means of evaluating the dyed protein fractions, has been examined more extensively, and the results of the investigation are presented in this paper. The adsorption of various dyes by protein on filter-paper and the

relationship between light absorption and concentration of dye adsorbed to protein have also been studied. In addition, an improved tank for paper electrophoresis has been devised and is described.

Experimental

Electrophoresis was carried out in a flat tank (Fig. 1) similar to that described by Franglen,[15] but incorporating several modifications, including a cooling system, based on that described by Boyd.[16] This prevents the heating effect of the electric current from causing excessive evaporation from the paper and also avoids the nuisance of water condensing on

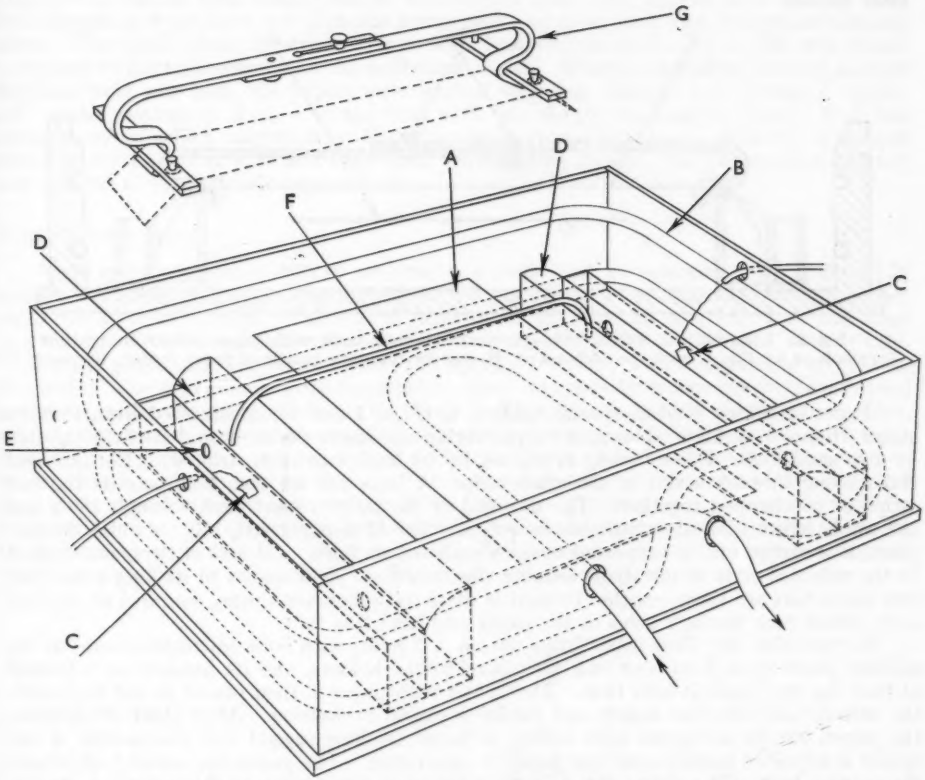

Fig. 1. Perspective of electrophoresis tank: A, copper sheet; B, cooling tube; C, platinum electrodes; D, troughs for buffer solution; E, hole in baffle plate; F, polythene siphon; G, holder for paper

the lid with which the tank is fitted and then dropping on to the paper. The walls and base of the tank (38 cm × 30 cm × 9 cm) are made of Perspex 9 mm thick, and a glass plate (40 cm × 30 cm × 0·6 cm) forms the lid. In the centre of the tank is a piece of copper sheet, A (25 cm × 15 cm × 0·1 cm), arranged crosswise horizontally (Fig. 2). To the underside of this copper sheet is soldered a loop of copper tubing (external diameter 5 mm, internal diameter 3 mm), one arm of which, B, extends to wind twice round the inner walls of the tank. Cold tap water (temperature 8° to 13° C, 500 ml per minute) is made to circulate through the tubing. (By adjusting the temperature of the circulating water the temperature of the tank could be controlled.)

Platinum electrodes, C (1 cm × 1 cm), attached to short lengths of platinum wire, dip into buffer solution contained in troughs, D (25 cm × 5 cm × 4 cm; capacity about 400 ml), constructed from Perspex 3 mm thick. These troughs can be removed from the tank for emptying and cleaning. A Perspex baffle plate perforated by two holes, E (diameter

5 mm), divides the trough into a small electrode chamber and a larger chamber into which dip the ends of the paper strips. In use, these holes are plugged moderately tightly with cotton-wool and, being below the surface of the buffer, allow electrical conduction between the chambers, although satisfactorily preventing any pH change produced at the electrode from being transmitted to the adjacent chamber. A thin polythene tube, F (internal diameter 1·5 mm), bent at right angles at each end (by heating), stretches as a siphon between the troughs and maintains hydrostatic equilibrium between them. The tube, which is easily filled with buffer solution by the use of a syringe and a bent intravenous needle, remains open while electrophoresis is in progress and passes only a negligible fraction of the total current.

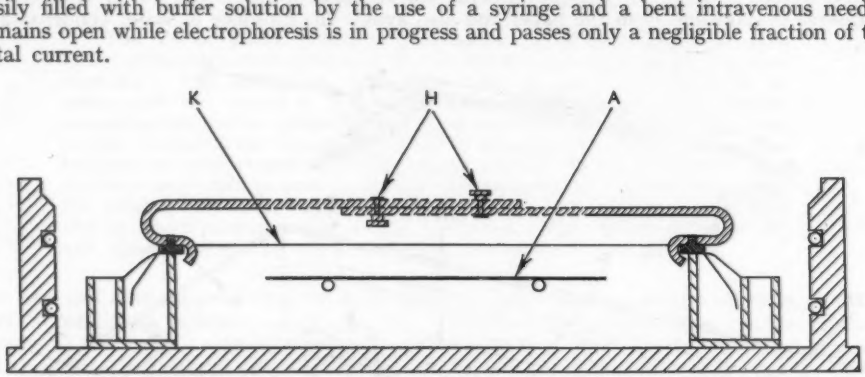

Fig. 2. Longitudinal section through electrophoresis tank with paper holder in position; symbols as for Fig. 1, with the addition of: H, nut for clamping handle of paper holder; K, paper

Paper strips are held in special holders, G (Figs. 1 and 2), constructed from Perspex strips (10 mm × 3 mm). Two such strips overlap one above the other and are held together by two small bolts, H, one being firmly set by its head into each strip, with the threaded end passing through a slot in the other strip. A large nut on each bolt permits the two strips to be clamped together. The free end of the strip is bent back through 180°, and the whole forms a handle adjustable in length. The filter-paper strip, K, on which electrophoresis is carried out, is supported towards each end on horizontal bars of Perspex attached to the reflected ends of the strips forming the handle, at right angles to its long axis. Set into these bars are short lengths (10 mm) of glass rod (diameter 4 mm), rounded at the free ends, which pass through holes in the paper and hold this taut.

In operation, dry filter-paper strips (30 cm × 3·8 cm) with holes (diameter 4 mm) in the midline about 4 cm from each end are placed in the holders, and its handles are adjusted so that the dry paper is held taut. The holder with paper is then placed in the tank with the ends dipping into the buffer, and the lid is placed in position. After about 30 minutes the paper, evenly saturated with buffer, is found to have sagged and the handle of the holder is adjusted further until the paper is again taut. The paper lies about 1 cm above the copper sheet. The serum (10 μl) is then run on to the surface of the strip in a narrow band across the paper at a point about 7 cm from the cathode end of the holder, leaving a clear band of paper (width about 5 mm) on each side. The lid is replaced and a further period of 60 minutes is allowed to elapse before the power supply is switched on. The tank will accommodate six strips (three holders). It has been found, however, an advantage to run only four separations and to set up a dummy strip on each side external to the test strips. With this arrangement a more even and parallel migration of the protein fractions is obtained.

The direct current used in this work was obtained from the a.c. mains by using a full-wave thermionic rectifier with choke - condenser smoothing. With the buffer solution described below, a potential gradient of 4 volts per cm gave a current of about 1·2 mA per strip and adequate separation of serum-protein fractions was obtained with this in 12 to 16 hours.

The buffer solution in this investigation was similar to that described by Michaelis.[17] The exact composition was: sodium diethylbarbituric acid, 5·00 g; sodium acetate trihydrate, 3·25 g; 0·1 *N* hydrochloric acid, 34·2 ml; water to 1000 ml; pH 8·6; I = 0·05. The filter-paper used was Whatman No. 3 MM. Fresh buffer solution was used for each run.

STAINING—

The dyes used were: (*i*) light green S.F. (Lissamine green S.F.130, Imperial Chemical Industries Ltd., Dyestuffs Division)—200 mg per 100 ml in 3 per cent. w/v aqueous salicylsulphonic acid[7]; (*ii*) bromocresol green (The British Drug Houses Ltd.)—200 mg dissolved in 20 ml of ethanol and made up to 100 ml with 3 per cent. w/v aqueous salicylsulphonic acid; (*iii*) naphthalene black (Amidoschwarz 10B, Bayer)—200 mg per 100 ml in 3 per cent. w/v aqueous salicylsulphonic acid.

After electrophoresis the papers were removed from the tank and placed directly into the combined staining and fixing bath, in which they were allowed to remain for 1 hour. After being stained with light green or bromocresol green, the papers were washed with four changes in 2 per cent. v/v aqueous acetic acid and then with two changes in distilled water. The final colour of strips stained with bromocresol green ($pK = 4{\cdot}6$) was found to depend on the thoroughness of the washing in water. When naphthalene black in salicylsulphonic acid was used, the papers were washed with four changes in a methanol - acetic acid - water mixture (4:1:5, v/v) and then with two changes in distilled water. In a few experiments in which naphthalene black was used the staining and washing technique described by Latner *et al.*[10] was employed. After being washed, the papers were blotted and allowed to dry at room temperature.

ELUTION OF DYES—

Light green was eluted with 40 per cent. v/v acetic acid, bromocresol green with 0·01 *N* sodium hydroxide and naphthalene black with 2 per cent. w/v sodium carbonate in 50 per cent. v/v aqueous ethanol. The optical densities of the resulting solutions were measured in a Unicam SP600 spectrophotometer at wavelengths of 630, 613 and 615 mμ, respectively. With light green and bromocresol green the uptake of dye by paper containing no protein was found to be negligible. With naphthalene black an appreciable amount of dye remained in the paper and allowance was made for this. In the analysis of serum proteins, the papers, after being stained, were cut transversely into strips containing individual zones, and these were dropped into measured volumes of eluting fluid. Sufficient eluting fluid was used to ensure that E_{1cm} did not exceed 0·8. In the analysis of serum the amount of dye adsorbed by a given protein zone as a percentage of the total dye adsorbed was taken as the estimate of the relative amount of protein in that zone. No correction was made for any difference in dye-binding capacities of different fractions, since it was held that there was no adequate standard on which to base correction factors, as will be discussed later.

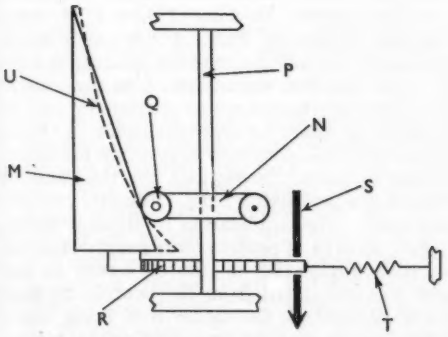

Fig. 3. Recording mechanism of reflectance densitometer. M, metal triangle; N, pen carriage; P, runner for pen carriage; Q, wheel; R, optical wedge filter; S, light-beam; T, spring (extension); U, outline of curved Perspex plate

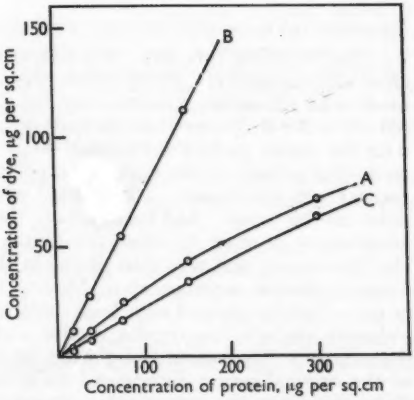

Fig. 4. Adsorption dye by serum protein precipitated on filter-paper. Protein was contained in circular areas approximately 16 mm in diameter. Dyes: A, light green; B, bromocresol green; C, naphthalene black

DIRECT PHOTOMETRY—

For scanning the dried stained papers a double-beam reflectance densitometer (Model 3 SR, Joyce, Loebl & Co. Ltd., Newcastle-upon-Tyne) was used, as supplied, and modified as described below. This instrument, which is based on that described by Latner *et al.*,[10] has a null-point indicator and employs twin light beams, one of which is reflected from the surface of the paper, whereas the other passes through an optical wedge filter. Attached to the optical filter is a triangular metal plate, M, so arranged (Fig. 3) that movement of a pen carriage, N, sliding along a fixed horizontal runner, P, and bearing by means of a wheel, Q, on the hypotenuse of the triangular plate, causes the metal plate to move and, consequently, the optical filter, R. Thus by moving the pen carriage the position of the optical filter may be adjusted so that the light beam, S, passing through it balances the light beam reflected from the surface of the paper. Displacement of the optical filter is directly proportional to (actually, $\frac{1}{3}$) the displacement of the pen, and, since the optical wedge filter is graded in a logarithmic manner, the instrument gives readings on a linear optical-density scale. The scanning beam measures 10 mm × 1 mm approximately. A blue-sensitive gas-photocell with a red filter (Ilford No. 204) was used for papers stained with light green or naphthalene black, and a blue-sensitive vacuum-photocell without filter was used for papers stained with bromocresol green.

EVALUATION OF ELECTROPHORESIS "DIAGRAMS"—

For this purpose the total area under the electrophoresis diagram must be subidvided into areas corresponding to protein fractions. Four methods of delineating these areas are available—

(*a*) subdivision of the total area by dropping perpendicular lines from the minima on the curve to the base line[5];
(*b*) subdivision of the total area into a series of peaks symmetrical about a perpendicular line dropped from the maximum to the base line[18];
(*c*) fitting of a series of Gaussian curves[19,20]; or
(*d*) subdivision of the total curve into triangles each corresponding to a protein fraction.

The first of these methods corresponds to the separation employed with elution and makes no allowance for overlapping of zones. The second and third methods make allowance for overlapping of zones, but are laborious and cannot be readily applied to asymmetrical peaks, *e.g.*, that of γ-globulin. The fourth method, which was that used in this investigation, makes some allowance for overlapping of zones, is easily applied to asymmetrical peaks and involves only simple arithmetic. The area of a triangle, as a percentage of the total area, was taken as an estimate of the relative amount of protein in the corresponding zone.

MEASUREMENT OF DYE UPTAKE BY PROTEIN ON FILTER-PAPER—

The following procedure was adopted as providing conditions nearest to those obtaining after electrophoresis. Fresh whole serum was used and serial dilutions (1/4 to 1/64) were made with the buffer solution used for electrophoresis as diluting fluid. Filter-paper strips (20 cm × 3·8 cm) were then sprayed lightly with buffer, avoiding complete saturation and, with the paper surface horizontal, 40 μl of each serial dilution were allowed to run slowly on to the surface of the paper, the point of the micro-pipette remaining stationary and in contact with the paper. The "spots" were sufficiently far apart for no overlapping to occur. After all the "spots" had been put on, the paper was allowed to remain in position for about 10 minutes, in order to allow the diluted serum time to spread throughout the thickness of the filter-paper, and was then placed in the combined dye and fixing bath. Roughly circular areas (diameter approximately 16 mm) were obtained. The application of diluted serum to paper lightly sprayed with buffer was found to give an area of protein more evenly stained, although slightly less regular in size and shape, than application of diluted serum to dry filter-paper. The amount of protein in each spot was calculated from the protein content of the original serum determined by a micro-Kjeldahl method, the factor 6·25 being used. From this figure the approximate concentration of protein in each pot was obtained by taking the area of the spots to be 2 sq. cm. For elution of the dye from these spots the paper strip was cut into segments of 2·5 cm long, each containing a dyed spot, and a segment of similar size not containing a dyed spot was taken to provide a blank. Before elution the strips were scanned with the JLC densitometer. Since the light beam in the latter covers an

area 10 mm × 1 mm and the diameter of the "spot" is 16 mm, the resulting curve consisted of a series of flat peaks.

MODIFICATION OF THE JLC REFLECTANCE DENSITOMETER*—

The mean readings corresponding to the flat peaks of triplicate spots of four serial dilutions of serum (1/8 to 1/64) were obtained. Since the displacement of the optical wedge filter is one-third of that of the pen carriage, the displacements of the former corresponding to each protein concentration were obtained by dividing the mean readings by 3. A graph was constructed on squared graph paper, relating displacement of optical filter to protein concentration (scales: ordinate, protein concentration 16 cm ≡ 150 μg of protein per sq. cm; abscissa, displacement of optical wedge filter 1 cm ≡ 1 cm). A piece of Perspex sheet (U, Fig. 3) 3 mm thick was cut to fit this curve and was then made to take the place of the original triangular plate. Displacement of the pen carriage was now proportional to the concentration of protein.

RESULTS

The optical densities of light green in 40 per cent. w/v acetic acid, of bromocresol green in 0·01 *N* sodium hydroxide and of naphthalene black in ethanolic sodium carbonate were found to be directly proportional to the respective dye concentrations provided $E_{1\,cm}$ did not exceed 0·8. The solutions of light green and bromocresol green were found to be stable

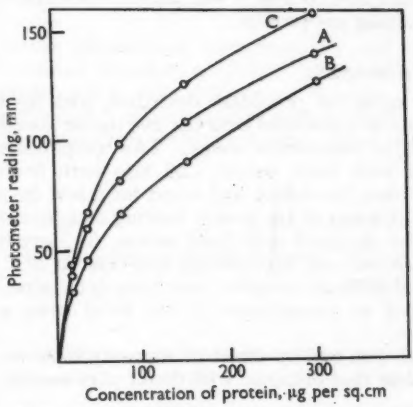

Fig. 5. Relationship between photometer reading and protein concentration. The protein was contained in circular areas approximately 16 mm in diameter and the photometer readings are those corresponding to the central portion of each area. Dyes: A, light green; B, bromocresol green; C, naphthalene black

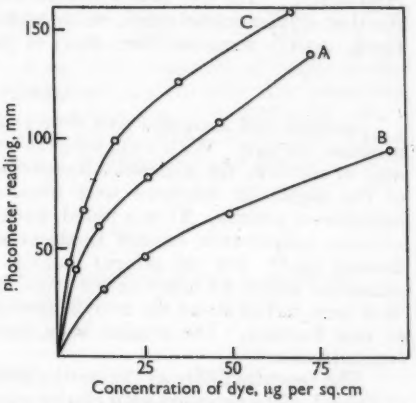

Fig. 6. Relationship between photometer reading and concentration of dye adsorbed on protein contained in circular areas of paper approximately 16 mm in diameter, the dye being eluted. The photometer readings are those corresponding to the central portion of each area. Dyes: A, light green; B, bromocresol green; C, naphthalene black

for at least 18 hours, this, for bromocresol green, confirming Franglen and Martin.[11] Naphthalene black in solution was found to fade slightly, a proportional reduction in optical density of about 7 per cent. at each concentration occurring in 18 hours.

With bromocresol green a direct proportionality between dye adsorbed and protein concentration was obtained with protein concentrations in the range 25 μg per sq. cm to 300 μg per sq. cm (Fig. 4). Below this range of concentrations the amount of dye adsorbed was relatively less. With naphthalene black the relationship deviated slightly from linearity at protein concentrations outside the range 25 to 150 μg per sq. cm. With light green the relationship deviated from linearity at all protein concentrations, although this was slight at protein concentrations up to 150 μg per sq. cm.

When the test strips were scanned, it was noted that the intensity of the dye was slightly greater on the surface of the paper to which the diluted serum had been applied than on the other surface. This difference was present even after some time had been allowed to elapse

* The possibility of such a modification was noted by Latner *et al.*[10]

between applying the diluted serum and staining the spots. Heating the strip at 110° C for 5 minutes after application of the serum but before staining did not eliminate the difference. A difference between the intensities of dyed protein zones on the upper and lower surfaces of the paper was noted frequently after electrophoresis—as will be discussed later in this paper. For the experiments here described, therefore, normally only the upper surface of the paper was scanned. The relationship between the photometer reading and the amount of protein present was examined by plotting the height of the flat peaks against the protein concentration (Fig. 5). A curvilinear relationship was found at all concentrations. The relationship between photometer reading and the amount of dye present, determined subsequently by elution, was also curvilinear (Fig. 6). A similar relationship was obtained with a calibration scale prepared from serum dilutions exactly as described by Latner *et al.*[10] Measurement of the amount of serum present per unit area of filter-paper revealed that, at any degree of serum dilution, the protein concentration per unit area was approximately 50 per cent. greater when calibration scales were prepared by the method of Latner *et al.* than when prepared by the method used in this work.

It was evident from these findings that, with the staining procedures employed, the unmodified photometer did not give readings directly proportional to the concentration of dye present or to the concentration of protein. However, modified as described above by the use of a curved Perspex plate in place of the triangular metal plate, it gave the desired relationship. The dye finally chosen was light green, because its colour did not depend, as did that of bromocresol green, on the amount of washing and because it was, unlike naphthalene black, readily removed from areas of paper containing no protein.

Analysis of serum protein

Normal and abnormal sera were examined by using the procedure described, with light green as the dye. With fresh sera the serum protein was resolved into the five major zones[2] and, in addition, the β-globulin fraction was resolved in two narrow zones. Two components of the β-globulin fractions were always obtained with fresh serum, and also with fresh heparinised plasma. It was noted, however, that when the serum had stood for a few days at room temperature, or at 5° C, the relative concentration of the slower moving component became less.[21] For the present purpose only results obtained with fresh serum, *i.e.*, serum examined within 48 hours of the blood being withdrawn and kept during this time at 5° C, have been included and the two components of the β-globulin complex have been determined as one fraction. The results have been expressed as percentages of the total protein concentration.

The reproducibility of the results obtained by elution and by direct photometry is shown in Table I. The precision with elution was greater than that obtained with direct photometry.

Table I

Analysis of a normal serum: reproducibility of results

Results expressed as means $\pm$ standard deviation of a single observation, in terms of percentage of total protein

Procedure	n	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
Elution, replicate strips	12	66·9 $\pm$ 1·75	4·4 $\pm$ 0·52	6·9 $\pm$ 0·55	9·2 $\pm$ 0·78	12·8 $\pm$ 1·21
Direct photometry, replicate strips	12	66·6 $\pm$ 2·53	3·9 $\pm$ 0·67	6·2 $\pm$ 0·77	7·6 $\pm$ 0·88	15·6 $\pm$ 2·11
Direct photometry, replicate scanning (one strip)	12	63·9 $\pm$ 0·64	3·7 $\pm$ 0·41	7·1 $\pm$ 0·32	7·1 $\pm$ 0·20	17·8 $\pm$ 0·53
Direct photometry, replicate planimetry (one curve)	12	63·6 $\pm$ 0·52	4·3 $\pm$ 0·42	7·1 $\pm$ 0·29	7·3 $\pm$ 0·15	17·7 $\pm$ 0·41

With the latter technique analysis of the precision obtained at each stage indicated that most of the error occurred in the electrophoretic separation and staining of the protein fractions, the least error being due to the actual photometry. Twofold dilution of the serum with buffer analysis was found to produce a small, but consistant, effect on the results obtained (Table II). With diluted serum the relative concentration of albumin was less than with undiluted sera, whereas the relative concentrations of the globulin fractions tended to be greater. The results obtained in analysing normal and pathological sera by means

TABLE II

ANALYSIS OF DILUTED AND UNDILUTED SERUM

Results expressed as means of duplicate analyses carried out on (*a*) undiluted serum, and (*b*) serum diluted with buffer solution (1 + 1); relative concentrations determined by elution and expressed as percentage of total protein

Serum		Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
A	*a*	67·2	4·6	7·5	9·5	11·3
	b	65·8	4·8	7·0	9·3	12·0
B	*a*	61·8	6·7	7·7	10·8	13·4
	b	58·3	7·1	8·7	9·5	16·4
C	*a*	43·8	5·3	12·1	10·3	28·5
	b	37·4	3·9	11·9	11·0	31·5
D	*a*	59·6	6·3	9·8	11·8	12·7
	b	56·7	8·5	9·8	10·9	14·1
E	*a*	67·0	3·2	6·2	9·7	14·2
	b	64·9	2·7	6·6	9·9	14·8
F	*a*	44·7	5·8	14·3	14·3	21·0
	b	37·3	9·3	18·6	15·7	19·1
Means	*a*	57·4	5·3	9·6	11·7	16·9
	b	53·4	6·7	10·4	11·5	18·0

of the photometer, both modified and unmodified, were then compared with the results obtained by elution (Table III). With the unmodified instrument (with the triangular metal plate), the differences between the relative concentrations determined by direct photometry and by elution were generally considerable, whereas with the modified instrument the differences were much less. An example of the effect of modification is given in Fig. 7.

TABLE III

COMPARATIVE ANALYSES OF VARIOUS SERA

Results expressed as mean differences ± standard deviation, or range, in terms of percentage of total protein

Method A	Method B	No. of sera	Results by method A *minus* results by method B: Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
Elution	Direct photometry, unmodified instrument ..	14	+17·4 ± 7·1	−1·2 ± 2·9	−6·0 ± 2·2	−7·7 ± 2·2	−3·4 ± 9·7
Elution	Direct photometry, modified instrument ..	45	−1·1 ± 3·8	+0·9 ± 1·9	−0·5 ± 2·2	+0·4 ± 2·2	+0·1 ± 2·7
Direct photometry, upper surface	Direct photometry, lower surface	17	+0·2 ± 4·1	−0·3 ± 1·2	+0·7 ± 1·0	−0·2 ± 1·9	−0·4 ± 3·8
Direct photometry	Direct photometry ..	17*	3·7 (0 to 8)	1·0 (0 to 2)	1·2 (0 to 4)	1·4 (0 to 3)	1·9 (0 to 7)
Elution	Elution ..	36*	2·3 (0 to 9)	1·4 (0 to 6)	1·1 (0 to 3)	1·4 (0 to 6)	1·6 (0 to 6)

* In these duplicate analyses all differences were taken as positive.

The height of the albumin peak is increased relative to the height of the lesser peaks. The results were as follows—

By direct photometry with the instrument unmodified: albumin, 33 per cent.; α_1-globulin, 9 per cent.; α_2-globulin, 17 per cent.; β-globulin, 18 per cent.; γ-globulin, 23 per cent.

By direct photometry with the instrument modified: albumin, 58 per cent.; α_1-globulin, 7 per cent.; α_2-globulin, 8 per cent.; β-globulin, 9 per cent.; γ-globulin, 17 per cent.

By elution: albumin, 55 per cent.; α_1-globulin, 9 per cent.; α_2-globulin, 10 per cent.; β-globulin, 10 per cent.; γ-globulin, 16 per cent.

A similar tendency is also apparent in the mean differences shown in Table III.

The differences between duplicate analyses on the same serum by direct photometry with the modified instrument ranged from a mean value of 1·0 for the α_1-globulin fraction to 3·7 for the albumin fraction (Table III). By elution the mean differences were of a similar magnitude. A difference in the photometer readings obtained by scanning upper and lower surfaces of the paper has already been mentioned. Generally the upper surface gave the higher reading; the mean ratio (upper to lower) of the readings corresponding to the albumin peak of 16 different sera was 1·14 (range 0·92 to 1·42). However, in spite of the differences

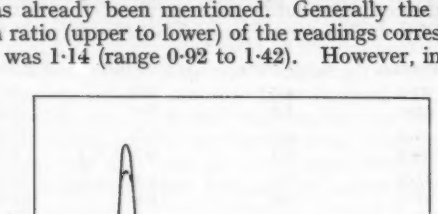

Fig. 7. Electrophoresis diagram of serum proteins. Continuous line: curve recorded with the modified photometer; broken line: curve recorded with the unmodified photometer

in the photometer readings of corresponding peaks, the relative concentrations of the protein fraction calculated from the curves obtained from each side of the paper were not appreciably different (Table III).

Discussion

The validity of the quantitative results obtained in the analysis of serum proteins by paper electrophoresis and measurement of dye uptake has recently been questioned.[11,22] Discrepancies between the results of serum-protein analyses obtained by the "free" electrophoresis procedure of Tiselius[23] and electrophoresis on paper have been noted by many workers,[2,3,24,25] and these discrepancies have been attributed, largely, to the differences noted in the dye uptake by individual protein fractions. Mackay, Volwiler and Goldsworthy,[26] however, have found good agreement between the results obtained by free electrophoresis and electrophoresis on paper. The heterogeneous,[27] and possibly variable, composition of the main protein fractions, however, makes it very difficult to obtain a standard on which to base comparisons. The specific refractive increments,[28] the relative nitrogen contents[28] and the dye-binding capacities[11,24,29] of individual proteins are known to differ from protein to protein, and for each protein fraction these properties can only be quantitatively constant if the composition of the fraction is constant. Until the composition of a fraction in terms of individual protein species is shown to be constant, the concentration of the main protein fractions cannot be determined in precise and absolute terms. However, the value of serum-protein analysis in the study of physiological and pathological processes does not depend entirely on the possibility of obtaining accurate absolute measurements of the various fractions present. What is required is a reasonably reproducible index of these fractions relative to each other or to the total amount of protein present. Changes in these indices then become of significance, even though the extent of these changes cannot be computed in precise terms. In this light the observed discrepancies between the results obtained by the "classical" method and those given by paper electrophoresis become unimportant, and it would not appear necessary for many purposes to make arbitrary corrections for any differences there may be in the dye-binding capacity of protein fractions.

In practice, the choice between two analytical procedures, assuming equal sensitivity and specificity, depends largely on the balance between the degree of reproducibility obtainable and the complexity of the technique employed. The complexity of replicate analyses by the "classical" electrophoresis method is certainly greater than that of paper electrophoresis.

With the "classical" method Armstrong[30] reported an experimental error in the resolution of schlieren diagrams of ± 1·5 per cent. of the total area. The over-all error in the analysis may well have been greater than this, since it is not clear whether the figures given refer to the whole process or only to the planimetry. Antweiler,[31] using a micro adaptation of the "classical" apparatus, reported a standard deviation of ± 0·5 per cent. of total protein for each component. The reproducibility of results obtained in this investigation (Table I), together with those obtained by other investigators using various methods for the electrophoretic analysis of serum proteins on paper,[5,12,26,32,33,34,35] indicate that the reproducibility obtained with paper electrophoresis and measurement of dye uptake is of the same order as that obtained with the "classical" method. The sensitivity of paper electrophoresis is actually greater than that of the "classical" method, whereas the specificity would appear to depend more on the conditions (pH, type of buffer solution, temperature and so on) obtaining during electrophoresis than on the method employed. On these grounds it would appear that paper electrophoresis with measurement of dye uptake may be accurately employed to measure serum-protein fractions in terms of a series of relative indices.

Certain points arising out of this investigation require further discussion. The results obtained indicate that dyes adsorbed by protein precipitated in filter-paper may be measured by direct photometry, with reflected light, and thus confirm the findings of Rottger[9] and Latner *et al.*[10] For the purpose of dye uptake by protein, direct photometry offers several advantages over methods involving elution in that it is less time-consuming and provides a permanent record of the spatial relationships of areas containing dye. Further, the strip itself may be preserved. The reproducibility obtained with elution (Tables I and III), however, is rather better than with direct photometry, and it has been noted also that with certain instruments and staining techniques the photometer reading (in terms of optical density of dye - protein complex) is not proportional to the dye concentration, which necessitates either the direct calibration of the instrument or the application of correction factors, *e.g.*, by the method of Crook *et al.*[5] The type of photometer used in this investigation is relatively easy to calibrate so that it gives readings directly proportional to the dye concentration, and a series of Perspex curves may be constructed for use with different staining techniques. Further, the determination of dye by means of an instrument measuring the reduction of reflected light avoids the need for rendering the paper translucent. The difference in the readings obtained in scanning the upper and lower surfaces is possibly due to a failure of the serum protein to permeate evenly through the thickness of the filter-paper, even in the 12 to 16 hours during which electrophoresis takes place. However, the agreement between the relative concentration of protein fractions calculated from the curves obtained from each side makes this finding relatively unimportant.

Latner *et al.*,[10] using a photometer similar to that used in the present investigation, reported that the photometer readings (in terms of optical density) were proportional to protein concentration. Present findings indicate that photometer readings (in similar terms) are not proportional to protein concentration, even when an exactly similar method of preparing standard curves is employed. The discrepancy between present findings and the conclusion of Latner *et al.*[10] is, however, more apparent than real, for inspection of the calibration data of these workers reveals that their scanner was less sensitive (presumably owing to the use of a different optical system) than that used in the present investigation, in which lower protein concentrations (amount of protein per unit area of paper) were found to give equivalent readings on the scanner. Examination of their published calibration curve shows that at the lowest four serum dilutions examined, which corresponded to the range covered by the instrument used in the present investigation, the plot of their experimental data is consistent with a curvilinear relationship between scanner reading and protein concentration, although, when the whole range of protein concentration is considered, the relationships do not deviate significantly from linearity.

The use of a combined fixing and staining bath[7] avoids the necessity for drying the strips after electrophoresis, a procedure that has been found to reduce the sharpness of the resolved protein zones. It would seem also that with this method the uptake of dye by protein is more nearly proportional to the protein concentration than has been reported for other staining techniques.[11] Martin and Franglen[11] and others[12,13] have noted differences also in the relative uptakes of dye by albumin and the main globulin fractions. In the present studies whole serum was used to investigate the dye uptake, since it was held that no advantage would be gained by applying corrections for any difference in dye uptake by

protein fractions, and, although linearity between dye uptake and a protein concentration could not be demonstrated with the dye used for all protein concentrations, deviations from linearity were not appreciable over a sufficiently wide range. The application of only 10 μl of serum per strip ensured that the protein concentration did not exceed this range (except rarely in the analysis of sera from patients with multiple myeloma, whose serum-protein pattern contained a relatively narrow and concentrated zone). The effect of dilution of the serum on the results (Table II) is probably due to the absence of exact proportionality between dye uptake and protein concentration over all ranges of the latter. However, the differences produced by a twofold dilution of the serum are so small that the changes in total protein concentration of serum occurring as a result of *in vivo* alteration in serum water content cannot appreciably affect the results obtained with a standard procedure.

Discombe *et al.*,[7] using light green as a dye and measuring the dye by direct photometry (transmission), have also noted that the results depend on the amount of serum analysed, the relative concentration of the largest protein fractions present (γ-globulin in the example quoted) being apparently less when a larger amount of serum was analysed. The differences noted were larger than those found in the present investigation and may possibly have been due, in part, to the absence of a linear relationship between scanner reading and protein concentration—a point on which no information is available.

It may be concluded, therefore, that with zone electrophoresis on paper and direct photometry of the stained protein zones, serum-protein fractions may be measured as accurately as with other electrophoretic techniques and with greater ease, so that changes in the distribution of serum proteins may be computed sufficiently accurately to provide useful information. Moreover, it must be emphasised that with no existing method of analysis can serum-protein fractions be measured in absolute terms.

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DEPARTMENT OF CLINICAL CHEMISTRY
UNIVERISTY OF EDINBURGH

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The Simultaneous Determination of Traces of Benzene and Toluene

BY W. HANCOCK AND E. Q. LAWS

A method is given for the quantitative evaluation of benzene and toluene, determined as *m*-dinitrobenzene and 2:4:6-trinitrotoluene, in mixtures in the range 0 to 50 μg of both nitration products.

METHODS have been published[1,2,3,4] for the quantitative determination of benzene in air in the presence of toluene and xylene by using the well known Janovsky reaction between *m*-dinitro aromatic bodies, a ketone and alkali. These methods rely on maintaining the colour due to *m*-dinitrobenzene and suppressing that due to the other interfering nitro compounds. Our previous work on this reaction[5] led us to study conditions of nitration and the resulting products. This paper is confined to the nitration of benzene and toluene in trace amounts and the quantitative evaluation of both constituents by using the Janovsky reaction, the optical density of the coloured solution being measured at two wavelengths. It is our intention to proceed to the study of the nitration products of the xylenes.

EXPERIMENTAL

NITRATION OF BENZENE AND TOLUENE—

The products of the nitration of benzene and toluene are affected by many factors and they have been the subject of exhaustive studies.[6,7,8,9,10,11] Some examples are given in Table I. From these data it is possible to select conditions of nitration that yield products suitable for our present purpose. In fact, we have chosen method No. 6, as it satisfies our requirements, giving a product consisting mainly of *m*-dinitrobenzene and 2:4:6-trinitrotoluene. It is clearly desirable that the formation of the two compounds in which we are interested should be at a maximum for both.

TABLE I

NITRATION PRODUCTS OF BENZENE AND TOLUENE

0·05 ml of each hydrocarbon taken in 10 ml of nitration acid

Method number	Nitration acid	Benzene	Toluene
1	Cold concentrated nitric acid, sp.gr. 1·42	Nitrobenzene, no *m*-dinitrobenzene	Nitrotoluene, no 2:4-dinitrotoluene
2	Hot concentrated nitric acid, sp.gr. 1·42	Nitrobenzene, no *m*-dinitrobenzene	Nitrotoluene, no 2:4-dinitrotoluene
3	Cold fuming nitric acid, sp.gr. 1·50	Nitrobenzene with a trace of *m*-dinitrobenzene	Nitrotoluene, some 2:4-dinitrotoluene, no trinitrotoluene
4	Hot fuming nitric acid, sp.gr. 1·50	Nitrobenzene with some *m*-dinitrobenzene	Some nitrotoluene, mostly 2:4-dinitrotoluene, no trinitrotoluene
5	Cold concentrated nitric acid, sp.gr. 1·42 - sulphuric acid (1 + 20)	*m*-Dinitrobenzene (trinitrobenzene is not formed)	2:4-Dinitrotoluene, no trinitrotoluene
6	Hot concentrated nitric acid, sp.gr. 1·42 - sulphuric acid (1 + 20)	*m*-Dinitrobenzene (trinitrobenzene is not formed)	2:4:6-Trinitrotoluene

With *m*-dinitrobenzene and 2:4-dinitrotoluene as starting materials, a range of experiments was carried out based on the data of Table I to find the conditions under which the production of 2:4:6-trinitrotoluene is at a maximum and the loss of *m*-dinitrobenzene and

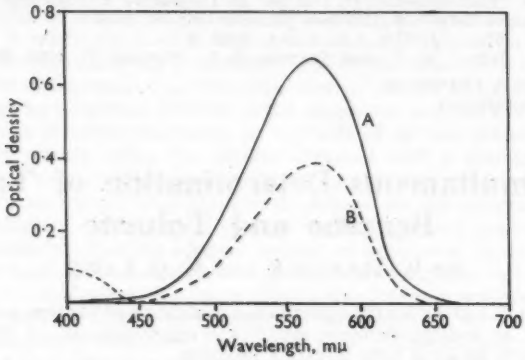

Fig. 1. Absorption spectra: curve A, *m*-dinitrobenzene; curve B, 2:4-dinitrotoluene

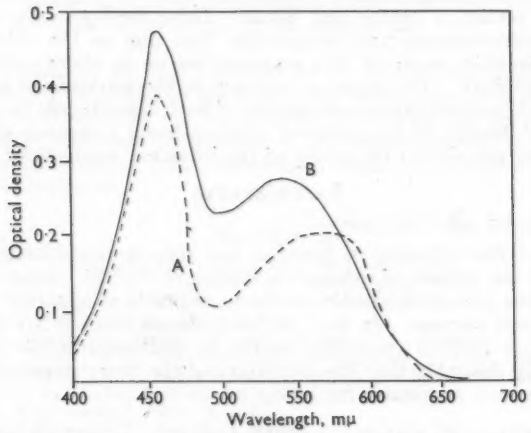

Fig.2. Absorption spectra: curve A, 1:3:5-trinitrobenzene; curve B, 2:4:6-trinitrotoluene

2:4-dinitrotoluene (and so trinitrotoluene) is at a minimum. Nitration for 2 hours at 105° C was finally selected. The difficulty is to complete the trinitration of the toluene and at the same time prevent the destruction by oxidation of the 2:4-dinitrotoluene and of the 2:4:6-trinitrotoluene formed. Under the experimental conditions selected the loss of 2:4:6-trinitrotoluene was about 20 per cent. and of *m*-dinitrobenzene about 6 per cent. These figures were obtained by the method described in this paper by comparing the recovery after nitration with results obtained with the pure compounds. As a consequence of these losses, it is necessary to prepare calibration curves for the two compounds under the experimental conditions.

If the starting materials are benzene and toluene, there is a further loss amounting to about 10 per cent. in each case, since the yields of *m*-dinitrobenzene and 2:4-dinitrotoluene are only about 90 per cent., owing to the formation of isomers. It is therefore necessary to construct practical benzene and toluene curves to correct for this factor. When 50 μg of benzene were taken, 43 μg were found—a recovery of 86 per cent.; when 19 and 27 μg of toluene were taken, 13 and 19 μg, respectively, were found—recoveries of 69 and 70 per cent.

THE JANOVSKY REACTION—

The Janovsky reaction[12,13] between dinitro compounds, ketone and alkali occurs most readily with nitro groups that are in the *meta* position to each other. In the range with which we are concerned (0 to 50 μg), we may regard *o*-dinitrobenzene, *p*-dinitrobenzene and 3:4-dinitrotoluene as giving no colour, although in far higher concentrations they give colours with butanone and alkali and more intense colours with acetone and alkali.[14]

Trace amounts of compounds having a *m*-dinitro grouping give the characteristic colours of the Janovsky reaction. The trinitro compounds also yield these colours, as they must have at least one pair of nitro groups in the *meta* position. *m*-Dinitrobenzene, 2:4-dinitrotoluene, 1:3:5-trinitrobenzene and 2:4:6-trinitrotoluene were examined and the absorption spectra of their coloured reaction products were plotted for the wavelengths 400 mμ to 700 mμ, a Unicam SP600 spectrophotometer being used. The results are shown in Figs. 1 and 2. It is seen that the pairs *m*-dinitrobenzene and 2:4-dinitrotoluene and 1:3:5-trinitrobenzene and 2:4:6-trinitrotoluene give similar curves, but with the maxima displaced slightly. Each pair differs considerably from the other, the trinitro compounds showing two absorption peaks, with the larger one at a lower wavelength than the maximum of the dinitro compounds. The curves shown were obtained on approximately 50 μg of each nitro compound.

TABLE II

AMOUNTS OF NITRO COMPOUNDS TAKEN AND FOUND

m-Dinitrobenzene, μg	2:4-Dinitrotoluene, μg	Equivalent 2:4:6-trinitrotoluene, μg	Optical density (*a*) at 462 mμ	Optical density (*b*) at 565 mμ	*m*-Dinitrobenzene found, μg	2:4:6-Trinitrotoluene found, μg
Series A—						
50	40	50	0·460	0·872	48	54
10	40	50	0·412	0·354	10	51
5	40	50	0·412	0·296	5	51
5	25	31	0·262	0·217	6	33
50	10	13	0·144	0·694	48	13
10	10	13	0·114	0·195	10	13
50	5	6	0·101	0·685	49	8
25	5	6	0·072	0·360	25	7
25	5	6	0·075	0·360	25	7
5	5	6	0·055	0·094	5	7
Series B—						
3	50	63	0·470	0·297	4	65
0	50	63	0·431	0·241	1	60
50	0	0	0·052	0·590	48	2
1	40	50	0·374	0·222	2	52
50	1	1	0·062	0·620	50	3

NOTE—The results for series A of the Table are obtained with the factors on p. 41; those for series B were obtained with a different ketone, the following factors being used—

		462	565
Wavelength, mμ			
Optical density equivalent to 1 μg	*m*-dinitrobenzene	0·00074	0·01224
	2:4:6-trinitrotoluene	0·00714	0·00373

From Table I it is seen that 1:3:5-trinitrobenzene is not formed in the ordinary course of nitration and that 2:4-dinitrotoluene goes mainly to 2:4:6-trinitrotoluene by method 6. We are left with *m*-dinitrobenzene and 2:4:6-trinitrotoluene for the quantitative measurement. Now these have absorption maxima at 565 mμ and 462 mμ, respectively. In addition, 2:4:6-trinitrotoluene has only 33 per cent. of the absorption of *m*-dinitrobenzene at 565 mμ, whereas *m*-dinitrobenzene has 10 per cent. of the absorption of 2:4:6-trinitrotoluene at 462 mμ.

There is, therefore, a good possibility of making an accurate determination of each in the presence of the other. A series of results is given in Table II, based on known quantities of *m*-dinitrobenzene and 2:4-dinitrotoluene, nitrated at 105° C for 2 hours, extracted, measured and calculated as described below. All the recoveries are reasonably quantitative.

TIME OF SHAKING—

The colour due to 2:4:6-trinitrotoluene comes to its maximum and begins to fade more rapidly than that due to *m*-dinitrobenzene. The maximum of 2:4:6-trinitrotoluene

is reached in 15 to 20 minutes and fading then occurs rapidly. *m*-Dinitrobenzene approaches its maximum in 20 minutes, but remains stable for 45 minutes. As a working compromise, we have adopted a shaking period of 15 minutes, followed by an interval of 5 minutes for transfer to the absorption cell and taking the reading. It is important that the conditions of shaking should be the same for both the sample and standard, as extremely vigorous shaking shortens the time of colour development for the nitro compounds studied.

Method

Reagents—

Nitration acid—Made from analytical-reagent quality nitric and sulphuric acids, mixed in the proportions 1 to 20 v/v.

Ether—Freed from peroxides and aldehydes by shaking with water, and then by saturated ferrous sulphate - 10 per cent. sulphuric acid until a sample of the ether gives no reaction with potassium iodide solution.

Butanone—Commercial samples of this material are liable to be impure. The substance should be fractionated with a good column and only the first four-fifths of the fraction boiling between 79° and 81° C should be used. Different commercial samples have been found to give different colorimetric figures, but a satisfactory sample should give a reading for optical density of not less than 0·24 when tested with 20 μg of *m*-dinitrobenzene and potassium hydroxide under the conditions described below. It is emphasised that considerable variations may be found and that a frequent check should be made on the material selected for use.

Sodium chloride, saturated solution—Prepared from analytical-reagent grade material.

Sodium hydroxide, 2 per cent. solution—Prepared from analytical-reagent grade material.

Potassium hydroxide, 40 per cent. solution—Prepared from analytical-reagent grade material.

Liquid paraffin, B.P.

Procedure—

Collect benzene and toluene or their nitro derivatives at room temperature in 10 ml of nitration acid. Heat to 105° C and keep at this temperature for 2 hours. At the end of this time remove the tube containing the acid from the heating-bath and cool it. Pour the contents of the tube into 50 ml of water and rinse with a further 50 ml of water. Transfer the aqueous solution to a separating funnel and extract with 50 ml of ether. Run the aqueous layer into a second funnel and extract with a second 50-ml portion of ether. Reject the aqueous layer, and wash the first ether extract with 30 ml of 2 per cent. sodium hydroxide solution. Transfer the alkaline solution to the second funnel and extract the ether with it. Reject the aqueous layer. Extract the ether layers separately with 30-ml portions of saturated sodium chloride solution. Run off the aqueous layers and transfer the ether layers with rinsing to a 250-ml conical flask fitted with a ground-glass joint. It is important that no drops of aqueous solution should enter the flask. Add 0·5 ml of liquid paraffin to the ether extracts. Connect the flask to a condenser and remove the ether by gentle distillation. Remove the flask from the heat-source while a few drops of ether remain and evaporate the remaining ether by means of a stream of air. Add 10 ml of purified butanone to the flask and 1 ml of 40 per cent. potassium hydroxide solution. Place on a mechanical shaker and shake for 15 minutes. Prepare an absorption cell (a 10-mm cell is convenient) by placing a few drops of the potassium hydroxide solution in the bottom. Place a suitable amount of the butanone solution in the cell and take optical-density readings at wavelengths of 462 mμ and 565 mμ. Do not allow more than 5 minutes to elapse between the end of shaking and taking the reading. Substitute the observed values for a and b in the equations and calculate the quantities x and y of *m*-dinitrobenzene and 2:4:6-trinitrotoluene.

Standardisation—

Prepare standard solutions of *m*-dinitrobenzene and 2:4-dinitrotoluene by dissolving 0·2 g of the compound in concentrated nitric acid. Dilute an aliquot with nitric acid to give a solution containing 200 μg per ml. This may be kept as a stock solution. Dilute 5 ml of the stock solution to 100 ml with concentrated sulphuric acid. This gives a solution containing 10 μg per ml in nitration acid. Take appropriate amounts of this solution and make up to 10 ml with nitration acid.

The dinitro compounds are quite stable in nitric acid, but they decompose in nitration acid. It is therefore necessary to make the final dilution into the sulphuric acid only when the solution is required for use and to discard any unused solution. Treat the solutions as described under "Procedure."

CALCULATION OF RESULTS—

From the standardisation figures the optical density equivalents of 1 μg of *m*-dinitrobenzene and 2:4:6-trinitrotoluene may be found for the two wavelengths 462 mμ and 565 mμ.

Values obtained with one batch of purified butanone were as follows—

Wavelength, mμ		462	565
Optical density equivalent to 1 μg	*m*-dinitrobenzene	0·00080	0·01320
	2:4:6-trinitrotoluene	0·00786	0·00437

If the optical density readings obtained with a mixture at the two wavelengths are a and b and the amounts of the two compounds are x and y μg (x = *m*-dinitrobenzene, y = 2:4:6-trinitrotoluene), then—

$$0{\cdot}00080\,x + 0{\cdot}00786\,y = a\ (462\ \text{m}\mu) \quad (1)$$

$$0{\cdot}01320\,x + 0{\cdot}00437\,y = b\ (565\ \text{m}\mu) \quad (2)$$

Solving these equations for x and y we obtain—

$$x = 78{\cdot}4\,b - 43{\cdot}6\,a \quad (3)$$

$$y = 131{\cdot}7\,a - 8{\cdot}0\,b \quad (4)$$

Substituting numerical values for a and b (optical densities in Table 1f), we obtain the figures for *m*-dinitrobenzene and 2:4:6-trinitrotoluene.

DISCUSSION OF RESULTS

The method is empirical in that the factors obtained may vary quite considerably from one batch of ketone to another.

It is probable that other workers will obtain equations with different numerical constants. Provided that the tests on samples and standards are done under identical conditions this should not invalidate the method. Losses due to parallel nitration reactions and oxidation are a constant proportion of the amounts present and will not affect results obtained when sample and standard are treated in the same manner. It is seen that the recoveries given in Table II are reasonably quantitative. There is a tendency for the *m*-dinitrobenzene figures to be low and for the 2:4:6-trintrotoluene figures to be high. This may be due to the experimental factors or to the equations obtained from them. Derivation of an "equation of best fit" gives results that are not so far different from those recorded as to justify the time spent on the calculation.

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DEPARTMENT OF THE GOVERNMENT CHEMIST
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June 27th, 1955

The Detection and Determination of Traces of Polynuclear Hydrocarbons in Industrial Effluents and Sewage

Part IV.* The Quantitative Examination of Effluents

By PHILIP WEDGWOOD and RONALD L. COOPER

A method is described for the determination of polynuclear hydrocarbons in effluents and the results are discussed. Discharges into the sewers of effluents containing oil are shown to be a greater potential source of these compounds than gasworks spent ammoniacal liquor, which makes only a small contribution to the quantities found in the sewage. Carburetted water-gas effluent is taken as an example of effluents containing gas oil that has been subjected to cracking in the gas-producing plant.

The method is applicable to other effluents containing either petroleum or tar oils.

The method for the detection of traces of polynuclear hydrocarbons has been described in the earlier papers of this series.[1,2,3] Full details of the method for the determination of the hydrocarbons on the microgram scale together with the precautions necessary to achieve accuracy have been described by Cooper.[4]

Peak heights are measured by using a base line, assuming that background absorption is linear over short ranges of wavelengths. The precautions include purification of solvents and taking measurements of peak heights at a fixed slit width in accordance with results from calibration curves, with an optical density not greater than 0·8.

Sewage works effluents

Procedure—

Extract 1 litre of effluent three times with chloroform. Filter the chloroform extract and then remove the solvent by careful evaporation. Remake the solution in a few millilitres of *cyclo*hexane. Chromatograph on a short alumina column (1 cm × 1 cm) and examine each eluate for specific hydrocarbons. For quantitative analysis make up individual eluates to 5 ml or 10 ml or, when the optical density is very low, suitably combine them. The lower limit of the determinations of pyrene in 1 litre of effluent is about 0·1 part per 1000 million.

Results—

The effluents of a number of sewage works have been examined, and hydrocarbons such as acenaphthylene, pyrene, alkylpyrene, fluoranthene and 3:4-benzpyrene have been found. Some results for pyrene, which has received particular attention, are summarised in Table I.

Discussion—

The results generally indicate a level of concentration of pyrene of the order of 1 part per 1000 million. Crude sewage is more difficult to analyse than the treated effluents, owing to interference from the large amount of suspended matter and other unoxidised material.

A series of samples taken at different stages of treatment in a sewage works, during steady fair weather conditions, will show a reduction in pyrene content from inlet to final outfall. In the cases of sewage works B and D, treatment of the sewage was less efficient than at works A and C.

The effect on the pyrene content of the final filtration through sand at sewage works C was small.

*For particulars of Part I of this series see reference list, p. 44.

PROCEDURE—

Acidify 10 to 100 ml of effluent with dilute sulphuric acid (5 to 10 per cent.) and extract at least three times with chloroform until the aqueous phase is clear and colourless. Filter

TABLE I

DETERMINATION OF PYRENE IN SEWAGE EFFLUENTS

	Sewage works	Pyrene, parts per 1000 million
A	Crude sewage	1·0
	Final effluent	0·36
B	(Dry weather)	
	Feed to filters..	1·55
	Final effluent	1·50
	(Wet weather)	
	Crude sewage	0·43
	Final effluent (*a*)	0·15
	Final effluent (*b*)	0·23
C	Effluent from filters	0·53
	Feed to sand filters	0·51
	Final effluent	0·45
D	Final effluent (*a*)	1·35
	Final effluent (*b*)	0·90
	Final effluent (*c*)	1·25

NOTES ABOUT THESE SEWAGE WORKS—

A—Receives very little trade effluent and no gas works effluent.
An efficient sewage works operating the double-filtration process in bacterial beds.

B—Receives trade effluents, including tannery waste and effluent from a bacon factory.
An overloaded sewage works operating the single-filtration process in bacterial beds.

C—Receives a high proportion of trade effluents, including less than 0·2 per cent. by volume of spent gas liquor.
A very efficient sewage works in which, after the usual sedimentation, the sewage is treated by the activated sludge process, followed by single filtration in bacterial beds, settlement of humus and final sand filtration.

D—Received some trade effluents, including about 0·7 per cent. of spent gas liquor and a similar proportion of carburetted water-gas plant effluent.
An overloaded and rather inefficient sewage works practising single filtration in bacterial beds. (Now closed down.)

the chloroform solution, evaporate carefully and prepare a solution in *cyclo*hexane. Chromatograph on an alumina column (6 to 10 cm × 1 cm). The hydrocarbons separate into between 10 and 40 eluates, which are examined and suitably combined for determination.

Complete separation of the individual compounds is not necessary to obtain reproducible results, as can be shown by combining the eluates and repeating the chromatography and determination several times. Consistent results are obtained, even though variations in the degree of separation may be appreciable.

RESULTS—

Some typical results are given in Table II.

TABLE II

TYPICAL RESULTS

Hydrocarbons	Gasworks D,* p.p.m.	Gasworks B,* p.p.m.
Anthracene	7·0	1·6
Pyrene	7·0	2·7
Fluoranthene	—	1·7
3:4-Benzpyrene	1·0	0·34
1:12-Benzperylene ..	0·4	0·14
Oil (total)..	350	660

* Letters indicate same towns as in Table I.

DISCUSSION—

The oil content of these particular effluents is relatively high and they would be submitted to further treatment before discharge to sewers by filtration through coke beds or in a specially designed plant. In the case of the effluent produced at gasworks D filtration reduced the concentrations of anthracene, pyrene and 3:4-benzpyrene to 0·2, 0·15 and 0·02 p.p.m., respectively. Hydrocarbons having 3 and 4 rings are present in greater concentrations than 5-ring and 6-ring compounds. The concentrations of hydrocarbons generally found are from 100 to 1000 times greater than are found in spent gas liquor effluents.

SPENT AMMONIACAL LIQUOR

PROCEDURE—

Extract 100 to 200 ml of effluent, made strongly alkaline (10 per cent. in potassium hydroxide), by repeatedly shaking with chloroform in the manner described in Part III of this series of papers. Subsequent procedure and the method of determination is similar to that already described for carburetted water-gas effluent.

The pyrene content is of the order of 10 parts per 1000 million, a recent determination giving a result of 11·5 parts.

DISCUSSION—

The hydrocarbon content of well settled tar-free spent gas liquor is very much lower than that of carburetted water-gas effluent. With pyrene contents as a basis for making comparisons, it is evident that discharges of spent liquor from a gasworks to the sewers in normal proportions would not wholly account for the presence of polynuclear hydrocarbons in the concentrations found in sewage. For example, 0·7 per cent. of spent liquor containing 11·5 parts per 1000 million would represent only 0·08 parts per 1000 million, whereas higher concentrations were found in places where gasworks effluents were not discharged, and indeed at sewage works A, where the sewage was almost wholly from ordinary domestic sources.

It is evident that the discharge of untreated carburetted water-gas effluents in a like proportion (about 0·7 per cent. by volume in the particular case of gasworks D) and of similar composition to the examples given could result in an appreciable addition to the concentrations of polynuclear hydrocarbons in the sewage. The pyrene results indicate that the rate of oxidation of hydrocarbons in the sewage works is slow, but it has been made clear already in Part II of this series that the more complex hydrocarbons appear to be retained in the sewage humus, which is removed by sedimentation before the treated effluent is finally discharged to a river.

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An Absorptiometric Method for the Determination of Beryllium in Beryl

By J. B. POLLOCK

A method is described in which beryl is decomposed by fusion with sodium fluoride and subsequent heating to fuming with sulphuric acid. The solution thus obtained is treated with tartaric acid before being made alkaline and diluted to a known volume, from which an aliquot portion is taken. *p*-Nitrobenzeneazo-orcinol is added and the optical density relative to a blank is measured on the Spekker absorptiometer.

The ethylenediaminetetra-acetic acid method for the gravimetric analysis of beryl, as hitherto used in the Uganda Geological Survey laboratory, is accurate but tedious. The absorptiometric method described in this paper is designed to speed up the determination of beryllium oxide without significantly sacrificing accuracy. It is based, after Stross and Osborn,[1] on the measurement of the optical density of the lake formed by *p*-nitrobenzeneazo-orcinol with beryllium in sodium hydroxide solution,[2] but unlike the method proposed by Stross and Osborn, neither separations nor corrections are required, nor are there any interferences when assaying the usual Uganda beryls.

Method

Apparatus—

(*i*) Hilger and Watts Spekker absorptiometer, fitted with a tungsten-filament lamp, Calorex heat absorbing filters and Kodak No. 4 green filters. Seven clean and dry 2-cm glass cells.
(*ii*) A 35-ml platinum crucible and lid.
(*iii*) Two 50-ml burettes; National Physical Laboratory grade A or equivalent.
(*iv*) One 100-ml burette; National Physical Laboratory grade B or equivalent.
(*v*) Calibrated flasks of 100 ml, 250 ml and 500 ml capacity; National Physical Laboratory grade A or equivalent.

Reagents—

Standard solution—Weigh 0·14 g of Johnson Matthey Specpure beryllium oxide into a 250-ml beaker, cover the beaker with a clock-glass and place it in the fume cupboard with the fan running. Slide back the clock-glass so as to uncover the spout of the beaker, pour in 100 ml of 5 per cent. sulphuric acid and digest on a sand-bath or hot-plate until the oxide has dissolved.

Cool the solution, transfer it to a 600-ml beaker, dilute it to about 250 ml and add 40 g of tartaric acid. When this has dissolved, add 27 g of solid sodium hydroxide in 9-g portions, cooling after each addition, and then make the solution just alkaline to methyl red test paper by carefully running in 50 per cent. sodium hydroxide solution drop by drop from a burette. Add exactly 10 g of solid sodium hydroxide, transfer to a 500-ml calibrated flask, cool and make up to the mark with water.

Blank solution—Dissolve 40 g of tartaric acid in about 250 ml of water. Add 10 ml of 50 per cent. sulphuric acid and 25 g of solid sodium hydroxide in portions as described above. When all has dissolved, make alkaline with 50 per cent. sodium hydroxide solution, add exactly 10 g of sodium hydroxide and make up to 500 ml in a calibrated flask.

Dye solution—Weigh 0·05 g of *p*-nitrobenzeneazo-orcinol into a 400-ml beaker, and add 2 g of sodium hydroxide and 200 ml of water. Stir the solution for about 15 minutes, using an electric stirrer.

If there appear to be any lumps in the solution, remove them by means of a rubber-tipped rod and stir again before filtering through a 15-cm Whatman No. 541 filter-paper.

Sample solution—Weigh 2·0 g of sodium fluoride into a 35-ml platinum crucible, melt it briefly over the blast Meker burner, remove the crucible from the flame and set it aside to

cool. Weigh exactly 0·5 g of sample into the crucible, and fuse the mixture for 5 to 10 minutes (not longer).

Place the crucible on asbestos gauze on a tripod in the fume cupboard, add 3 to 3·5 ml of concentrated sulphuric acid, replace the lid and heat very gently until all effervescent action is finished, and then more strongly until fumes of sulphur trioxide are evolved. Complete the decomposition with 5 minutes' strong heating to ensure that all hydrofluoric acid is driven off. Allow the crucible to cool, place it in a 400-ml beaker and add about 150 ml of water. Warm the beaker on the hot-plate until solution is complete, and then remove it and rinse the crucible and lid.

Add 20 g of tartaric acid. When this has dissolved, add 12 g of sodium hydroxide in two portions, cooling after each addition, and make alkaline with 50 per cent. sodium hydroxide solution. Add 5 g of sodium hydroxide and make up to the mark in a 250-ml calibrated flask.

PROCEDURE FOR DETERMINING BERYLLIUM OXIDE—

Take seven 100-ml flasks and mark them 2, 4, 6, 8, 10, B and S. Into the first five add from a 50-ml burette, respectively, 2 ml, 4 ml, 6 ml, 8 ml and 10 ml of standard solution. Make the first four up to 10 ml with blank solution from a second burette. Into the flask marked B run 10 ml of blank solution and into S put, by pipette, 10 ml of sample solution.

Add to each flask 20 ml of dye solution from the 100-ml burette, and dilute the solutions in the flasks to the mark with water. Shake well, and to ensure thorough mixing pour the contents of each flask into a dry 250-ml beaker, and then back into the flask.

Measure the optical densities of the sample and standard solutions relative to the dye - blank solution in flask B, on the Spekker absorptiometer, using the tungsten-filament lamp, Kodak No. 4 green filters and 2-cm cells. Construct a calibration curve of optical density against beryllium oxide content expressed as percentage of beryllium oxide in a 0·5-g sample, the standard solutions representing 2·8 per cent. (2 ml), 5·6 per cent. (4 ml), 8·4 per cent. (6 ml), 11·2 per cent. (8 ml) and 14 per cent. (10 ml). The points may be joined with straight lines. Read off the beryllium oxide content of the sample.

INTERFERING ELEMENTS

Copper to a marked degree and zinc to a lesser extent form coloured lakes with *p*-nitrobenzeneazo-orcinol under the same conditions as beryllium, but since both these elements are absent from the usual Uganda beryls their interference is disregarded. If necessary, they can, however, be removed as sulphides by means of hydrogen sulphide.

Beryl itself may contain, apart from the beryllia, alumina and silica of its theoretical composition, subordinate down to trace amounts of iron, titanium, manganese, calcium, alkalies and so on, all of which can be substantially increased by any gangue rock overlooked when upgrading a beryl concentrate for sale. None of these elements interferes in the sense of forming coloured lakes with the dye, and those giving colourless solutions are wholly without influence on the course of the analysis.

Iron and manganese alone give coloured solutions. The former, in a concentration equal to 100 per cent. of ferric oxide in a 0·5-g sample, was found to interfere by an optical density of 0·010. As the poorest beryl concentrate does not contain more than 10 to 15 per cent. of ferric oxide, the influence of iron may thus be ignored.

Manganese in the initial sample solution gives a reddish-brown colour not unlike the beryllium lake, and might at first be supposed to interfere more severely than iron. To check on this point, two solutions were prepared in accordance with the instructions contained in the method, each containing beryllium equivalent to 14 per cent. of beryllium oxide, and one having, in addition, the manganese equivalent of 5 per cent. of manganese oxide. When measured against the usual dye - blank solution, the optical density of the solution containing manganese was only 0·010 greater than the plain beryllium solution. The manganese content of even a very impure beryl concentrate will not usually exceed 1 to 2 per cent. of manganese oxide at the most, so that the interference of this element also may be ignored.

In any event the presence of excessive quantities of manganese will be detected immediately from the colour of the initial sample solution; this colour will fade away wholly or partly, depending on the manganese concentration, after standing for 24 to 48 hours in a stoppered flask. If time permits, the interference of excessive amounts of manganese may

thus simply be eliminated, as a solution can be made up and used before atmospheric oxidation causes a return of the colour.

Experimental

The colour intensities of the dye - blank, dye - standard and dye - sample solutions, and the optical densities of the last two relative to the first, are dependent on the following factors.

Sodium hydroxide concentration—

The sodium hydroxide concentration, which governs the pH of the measurement solutions, influences the colour intensity of the dye in blank, standard and sample solutions, and to a lesser degree, that of the beryllium lake also.

The curve in Fig. 1 shows the sodium hydroxide content of the initial solutions required to give maximum relative absorption in the measurement solutions. The concentration is not critical between values of 1 and 2·5 per cent. w/v, but the figure of 2 per cent. was selected for use in an analysis as it permits an error of $\pm 0{\cdot}5$ g per 100 ml in weighing the final addition of solid alkali.

Concentration of dye solution—

Fig. 2, curve A, is a typical calibration curve obtained by following the prescribed method. It falls away progressively with increasing beryllium oxide content of the measurement solutions from the theoretical straight line of Beer's law.

The most probable explanation lies in the small excess of dye over beryllium in the solutions containing the higher quantities of beryllium oxide, for the maximum theoretical dye concentration is 5 mg per 100 ml, whereas the beryllium oxide content goes up to 2·8 mg per 100 ml. Unfortunately, it is not practicable to add more dye, since its intense colour results in excessive light absorption in the blank solution, with consequent loss of sensitivity, long before a sufficient concentration is reached to make the calibration curve straight.

This defect, inherent in the use of *p*-nitrobenzeneazo-orcinol, can be overcome by adding a large excess of sodium hydroxide. The excess of alkali so deepens the colour of the dye unconsumed by the lake formation that the optical density of the dye (as distinct from the lake colour) in all the standard solutions becomes equal within the perception of the Spekker

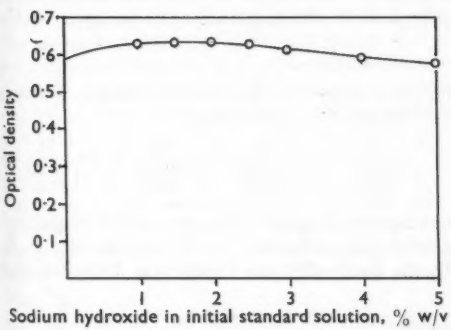

Fig. 1. Absorptions of beryllium - *p*-nitrobenzeneazo-orcinol solutions containing various amounts of sodium hydroxide; beryllium content equivalent to 14 per cent. of beryllium oxide in a 0·5-g sample

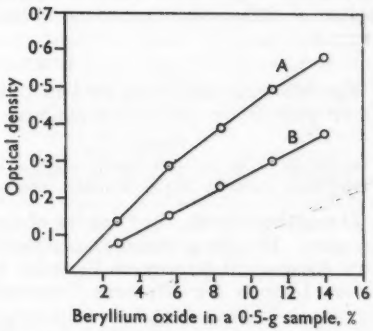

Fig. 2. Calibration curves: A, beryllium - *p*-nitrobenzeneazo-orcinol solution; B, beryllium - *p*-nitrobenzeneazo-orcinol solution containing excess of sodium hydroxide

absorptiometer. Such a remedy is not, however, satisfactory, as is shown by Fig. 2, curve B. The calibration curve becomes a straight line, but the relative optical density of the standard to the blank is greatly reduced, resulting in a shallow gradient and loss of sensitivity.

Tartaric acid concentration—

Fig. 3, curve A, shows another typical calibration graph, prepared by the prescribed method. Curve B is obtained by doubling the concentration of tartaric acid (made just

alkaline with sodium hydroxide) in the measurement solutions, other reagents being the same as for curve A. It will be seen that some care must be taken when weighing out the tartaric acid used to prepare the initial blank, standard and sample solutions in order that the concentration of this reagent may be substantially the same in all three.

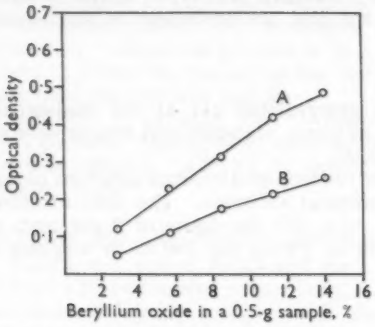

Fig. 3. Calibration curves: A, beryllium - *p*-nitrobenzeneazo-orcinol solution; B, beryllium - *p*-nitrobenzeneazo-orcinol solution containing double amounts of tartaric acid as sodium tartrate

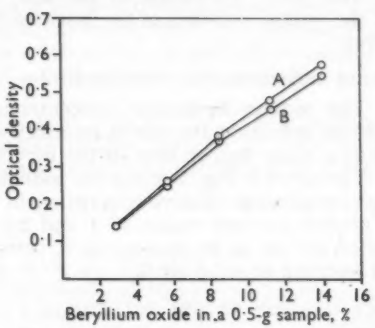

Fig. 4. Calibration curves for beryllium - *p*-nitrobenzeneazo-orcinol lake: curve A, measurements made within 30 minutes of preparing the solutions; curve B, measurements made on the same solutions after 48 hours' standing

It should be noted that not less than 8 per cent. of tartaric acid is necessary in the initial solutions to prevent the formation of an insoluble precipitate on standing.

TIME OF STANDING—

The colour of the beryllium - *p*-nitrobenzeneazo-orcinol lake develops immediately on adding the dye to the standard or sample solution, with little subsequent change. Curve A, Fig. 4, shows the absorptions measured within 30 minutes of preparing the solutions; curve B is for the same solutions measured 48 hours later.

A delay of a few hours in measuring the densities of sample or standard solutions is therefore of little significance, *e.g.*, solutions prepared in the morning may be used in the afternoon.

COMPARISON OF RESULTS

The following results are for four Uganda beryls analysed (*a*) by the gravimetric Versene method[3] and (*b*) by the absorptiometric method described in this paper—

Sample	A	B	C	D
Beryllium oxide by gravimetric method, % ..	9·66	10·87	11·14	11·20
Beryllium oxide by absorptiometric method, % ..	9·6	10·8	11·0	11·3

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GEOLOGICAL SURVEY OF UGANDA
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May 18th, 1955

The Elimination of Interference by Arsenic in the Polarographic Determination of Antimony and Tin

By G. PACKMAN and G. F. REYNOLDS

The development of methods for the elimination of the interference of arsenic in the polarographic determination of antimony and tin in sulphuric acid solution is described. Comparative results are presented.

Recently it became necessary to develop a polarographic method for the determination of microgram amounts of antimony and tin in aqueous solution. The method developed involved the polarography of antimony in 0·5 *M* sulphuric acid, after which the solution was made 6 to 7 *M* with respect to hydrochloric acid and a polarogram for tin was made. The existence of the antimony in the tervalent state was ensured by precipitating the sulphide from sulphate solution with hydrogen sulphide and evaporating until the sulphide redissolved.

In general, the results obtained were good, but in a number of cases it appeared that the antimony content was unexpectedly low, whereas that of tin was impossibly high. This was traced to the presence of arsenic in the samples, and it was further shown that the effect was serious only when the concentration of the arsenic approached that of the antimony. The work described in this paper was therefore undertaken to eliminate this interference with the determination.

Experimental

All polarography was carried out at 25° C on a Cambridge Instrument Co. photographic polarograph. All potentials are expressed in the European sign convention. In this convention the potential of the saturated-calomel electrode is taken as +0·246 volt against the normal hydrogen electrode.

Polarography of antimony in the presence of arsenic—

Studies of the polarographic characteristics of antimony in the presence and absence of arsenic and of the polarographic characteristics of arsenic itself were made. It was found, in agreement with some published work,[1,2] that quinquevalent arsenic is not reducible at the dropping-mercury electrode under the conditions that we used. The reduction wave for quinquevalent arsenic reported by Meites[3] was not obtained, since our experiments were carried out in 0·5 *M* sulphuric acid, whereas Meites employed solutions of a high chloride content. Tervalent arsenic, however, was shown to give a smoothly sloping line instead of a step of normal shape. This slope became perceptible at about the same applied potential as that of the beginning of the antimony step and maintained an almost constant value until a little after the completion of this step. A rapid non-linear increase in current then took place, the commencement of which was displaced towards less negative potentials with decrease in pH. Hence, the polarogram of antimony in the presence of tervalent arsenic consisted of a step with a considerable slope of the plateau, as shown in Fig. 1. A decreased value for the step height was therefore obtained by the normal extrapolation method (FG in Fig. 1).

In view of the fact that, for satisfactory polarography, it was necessary to retain the antimony in the tervalent state,[4] it was not possible to remove this arsenic interference by oxidation to the quinquevalent state. It appeared, however, that it might be permissible to apply a correction to the antimony step height if the arsenic polarogram could be regarded as a gradient linearly related to concentration rather than as a step.

A series of solutions of tervalent arsenic in 0·5 *M* sulphuric acid was therefore prepared and polarograms were recorded. The concentration range was 0 to 100 μg of tervalent arsenic per ml. The steady rise, already described, was obtained in each case and the vertical height of the rise between −0·40 and −0·65 volt (against the mercury pool) was measured. These values, after conversion to a constant polarographic sensitivity, were plotted as arbitrary scale divisions, against arsenic concentration. As shown in Fig. 2, the relationship was fairly linear, and so a mean factor for the relation between height of rise and concentration of arsenic was obtained.

A series of solutions containing known amounts of tervalent antimony and arsenic, within the concentration range 2 to 50 μg per ml, in 0·5 M sulphuric acid was next prepared and polarograms were recorded against the mercury pool. It was found that slight

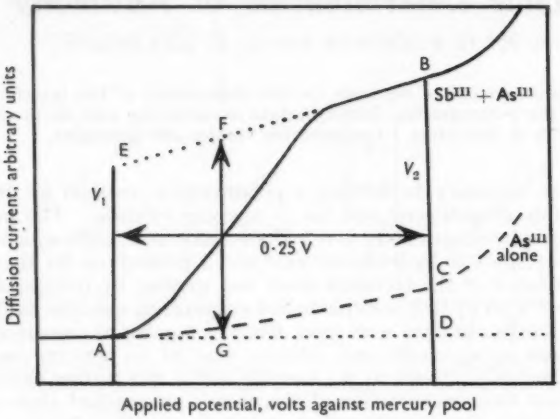

Fig. 1. Combined step for antimony and arsenic

changes in the half-wave potential of the antimony step occurred, and the position of V_1 (Fig. 1) was therefore taken as the foot of the antimony step, rather than a fixed point on the voltage axis; V_1 and V_2 were taken at 0·25 volt apart, so that the whole of the antimony reduction was included without inclusion of the subsequent steep rise. Counter-current settings were adjusted in each case to ensure that the foot of the antimony step was parallel to the voltage axis.

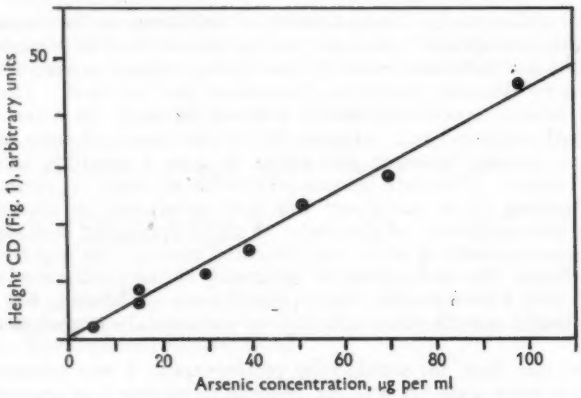

Fig. 2. Correction graph for antimony determination

The antimony recoveries were calculated from the step heights obtained, a comparison being made between the conventional method of measurement (FG in Fig. 1) and the corrected step height (BC in Fig. 1) after allowance for the contribution due to arsenic had been made, the height CD being found from Fig. 2. These recoveries are presented comparatively in Table I.

POLAROGRAPHY OF TIN IN THE PRESENCE OF ARSENIC—

In the medium employed for the determination of tin (3 ml of 0·5 M sulphuric acid mixed with 2 ml of 10 M hydrochloric acid) quinquevalent arsenic was again shown not to be polarographically reducible. Tervalent arsenic, however, gave a well defined step

TABLE I

POLAROGRAPHIC DETERMINATION OF ANTIMONY

Antimony added, μg per ml	Arsenic added, μg per ml	Antimony recovered by conventional method, μg per ml	Antimony recovered by correction method, μg per ml
2·0	2·0	1·6	2·0
2·0	5·0	1·3	2·3
2·0	5·0	1·6	1·9
2·0	10·0	1·1	2·4
5·0	20·0	3·8	4·9
10·0	40·0	6·3	9·8
30·0	30·0	25·0	29·1
30·0	30·0	21·5	30·8
30·0	50·0	23·0	29·6

almost coincident with that of tin. Since it was known that a well defined step at −0·47 volt, corresponding to the reduction—

$$Sn^{II} \rightarrow Sn(Hg)$$

is obtainable equally well from stannous or stannic solutions in this medium, it was considered that the interference could be removed by a suitable oxidation procedure.

A series of solutions of tin and tervalent arsenic in 6 ml of 0·5 *M* sulphuric acid was prepared, within the concentration range 10 to 60 μg per ml. To each solution 4 ml of 10 *M* hydrochloric acid and 0·2 ml of saturated potassium chlorate were added. The solutions were boiled until the odour of chlorine was absent. This was usually so when the volume had been reduced to about 7 ml. After cooling, the solutions were transferred to 10-ml calibrated flasks, and after treatment with 0·2 ml of 0·1 per cent. proteose peptone were made up to volume with water.

For purposes of comparison a similar series of solutions was prepared, in which the chlorate oxidation stage was omitted.

The recoveries of tin obtained by polarography of these solutions are given in Table II.

TABLE II

POLAROGRAPHIC DETERMINATION OF TIN

Concentration of tin, μg per ml	Concentration of arsenic, μg per ml	Recovery of tin with oxidation omitted, μg per ml	Recovery of tin after oxidation, μg per ml
10	0	10·0	—
20	0	20·5	—
20	20	—	20·0
20	30	45·0	—
20	40	—	20·5
30	0	30·0	—
30	30	56·0	28·5
30	60	82·0	29·5
40	30	71·0	—
60	30	94·0	59·5
60	60	—	60·5

DISCUSSION OF RESULTS

The results obtained for the determination of antimony show that within the concentration range investigated the correction for the effect of arsenic on the antimony step is accurate and reproducible. An independent determination of the arsenic concentration is, of course, necessary, and this may be made by any convenient method, such as that described by Sandell.[5] No investigation was made of the possible effects of the presence of other ion species on this determination, as we separated the antimony and tin from other constituents by co-precipitation on manganese dioxide.[6,7] The presence of manganese did not affect the determination, but it is thought that, in general, the correction procedure would be very susceptible to interference.

Acknowledgment is made to the Chief Scientist, Ministry of Supply for permission to publish this paper. Figs. 1 and 2 are reproduced by permission of the Controller H.M.S.O.

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CHEMICAL INSPECTORATE
MINISTRY OF SUPPLY
STATION APPROACH BUILDINGS
KIDBROOKE, LONDON, S.E.3

March 16th, 1955

Symposium on Gas Chromatography

(Report of a Joint Meeting of the Physical Methods Group, Microchemistry Group and the Scottish Section on Friday, May 20th, 1955)

ABOUT 130 analytical chemists were present at Imperial Chemical Industries' Works at Stevenston, Scotland, on Friday, May 20th, 1955, for the Symposium on Gas Chromatography, arranged jointly by the Physical Methods and Microchemistry Groups with the Scottish Section. All the visitors were taken on a tour of the Works and Research Laboratories and enjoyed the generous hospitality of I.C.I., who provided all necessary transport and meals for everyone. A memorable day was concluded with an excellent dinner for fifty in Glenfoot House, and about twenty other guests were entertained to dinner in West Kilbride.

The Symposium comprised two sessions in the Ardeer Recreation Club. In the morning the President of the Society, Dr. K. A. Williams, B.Sc., F.R.I.C., A.Inst.P., M.Inst.Pet., took the Chair, and the following papers were presented and discussed: "Gas - Liquid Chromatography," by A. J. P. Martin, Ph.D., F.R.S., Nobel Laureate; "The Vapour-phase Chromatographic Analysis of Hydrocarbon Mixtures," by D. E. Chalkley, B.A., B.Sc.; "Techniques used in a Study of the Boron and Silicon Hydrides," by A. B. Littlewood, B.A.

During the afternoon the visitors were conducted round the Stevenston Works, careful planning by the hosts ensuring that the five parties into which the visitors were divided saw displays of the applications of explosives and demonstrations of gas-chromatographic apparatus before being brought together again for the evening session. The apparatus used by the authors of the papers was demonstrated in the Research Laboratories; instruments developed by members of I.C.I. research teams and some industrial apparatus exhibited by manufacturers were on view in the Showroom.

Dr. D. Traill, B.Sc., F.T.I., F.R.I.C., Research Manager of I.C.I. Nobel Division, occupied the Chair for the evening session, which included the following papers: "Adsorption and Partition Methods," by C. S. G. Phillips, M.A.; "A Rapid Chromatographic Method for the Determination of Bromine-inert Impurities in Ethylene," by N. H. Ray, B.Sc., A.R.I.C.; and a discussion on "Katharometers as Recorders in Gas Chromatography," which was opened by Dr. Keulemans of Koninklijke/Shell-Laboratorium, Amsterdam, Holland.

The papers and discussions are summarised below.

GAS - LIQUID CHROMATOGRAPHY

DR. A. J. P. MARTIN gave a general review of the work he and Dr. A. T. James had done since 1950 on gas - liquid chromatography. Although the general idea for this method was suggested by Synge and himself in 1941, until 1950 the subject had been entirely neglected. Then James and he studied the separation of fatty acids and amines by the use of automatic titration apparatus and proved the general usefulness of the method, making simple extensions of theory to cover the differences between liquid - liquid and liquid - gas chromatography. In the range of conditions covered by their work, the efficiency of columns four feet in length varied from 500 to 2000 theoretical plates, efficiency increasing as the rate of flow of gas decreased and as the temperature was raised. In general, however, as the temperature was raised, the ratio of volatilities of a given pair in a homologous series decreased, so that a

better separation was usually obtained at lower temperatures than at high in spite of the lower efficiency of the column.

Except for the first two or three members of a series, the factor of increase of retention volume (*i.e.*, the volume of gas passed through the column before the peak emerged) was a constant for each additional $>CH_2$ group in the molecule. Hence plotting the logarithm of the retention volume against the number of carbon atoms in compounds of a given series led to a straight line. The slope of the line was very similar for a number of very different liquids used as the stationary phase of the column. But the ratio of retention volumes for substances possessing different types of groups (*e.g.*, –OH, $-NH_2$, >CO and CH_3CO–) was very different for these different phases; it was thus possible, if retention volumes of a few different columns (with different stationary phases) were known, to say much about the chemical nature of an unknown substance.

The merits of various current methods of detecting vapours in a permanent gas were briefly discussed and a description was given of the author's continuously recording gas-density meter. Several examples of the separation of different classes of substances were shown. In general, samples of a few milligrams sufficed for even very complicated mixtures; a few micrograms of single substances could be determined in favourable circumstances. There seemed to be no reason why quantitites of up to 10 g of pure substances could not be prepared with the resources of a normal laboratory. For larger amounts, however, the equipment became rather bulky and inconvenient.

The time taken for analysis seldom exceeded a few hours and was often only a few minutes, depending on the difficulty of the separation. Dr. Martin expressed the opinion that the method was destined to replace analytical procedures at present involving fractional distillation at pressures exceeding a few millimetres of mercury.

The method provided perhaps the easiest of all means of studying the thermodynamics of the interaction of a volatile solute with a non-volatile solvent, and its potential value for providing this type of data should be very great.

Discussion

Dr. E. F. G. Herington said that Dr. Martin had referred to thermodynamic relationships and that he proposed to discuss one particular thermodynamic method of considering gas chromatography. It was convenient to think of this technique as a form of extractive distillation. Expression (1) could then be derived by an argument similar to that previously employed for extractive distillation (Herington, *Research*, 1950, **3**, 41), remembering that most gas-chromatographic separations were carried out with a large excess of the immobile phase—

$$\log_{10} V_{21} \simeq \log_{10} (p_1^\circ/p_2^\circ) + X - Y \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where—

$$X = \Delta G^E_{N_1N_3'}/2{\cdot}303\,\boldsymbol{R}T\,(1 - N_3') \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$Y = \Delta G^E_{N_2N_3''}/2{\cdot}303\,\boldsymbol{R}T\,(1 - N_3'') \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Here—

V_{21} was the relative retention volume of component 2 divided by that of component 1,
p_1°, p_2° were the vapour pressures of pure components 1 and 2 at the absolute temperature T,
component 3 was the stationary phase,

$\Delta G^E_{N_1N_3'}$ was the excess free energy of mixing of the binary mixture containing N_1 mole fraction of component 1 and N_3' mole fraction of component 3 at the temperature T,

$\Delta G^E_{N_2N_3''}$ was the excess free energy of mixing of the binary mixture containing N_2 mole fraction of component 2 and N_3'' mole fraction of component 3 at the temperature T, and
$\boldsymbol{R}$ was the gas constant.

In the derivation of this expression the vapours were assumed to behave as ideal gases and the "approximately equal to" sign was used because expression (4) had been employed instead of equation (5).

$$V_{21} \simeq \alpha_2/\alpha_1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

$$V_{21} = (m + \alpha_2)/(m + \alpha_1) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

Here—

α_1 was the partition coefficient for component 1 and was defined as—

$$\frac{\text{concentration of solute 1 in non-mobile phase, g per ml}}{\text{concentration of solute 1 in mobile phase, g per ml}}$$

at equilibrium,

the partition coefficient for component 2 was similarly defined, and

m was the area of the cross-section of the mobile phase divided by the area of the cross-section of the stationary phase.

Expression (4) was a good approximation to (5) in practice, because for a good separation it was essential that $|m| \ll \alpha_1$ and $|m| \ll \alpha_2$.

The right-hand side of expression (1) contained three terms. The term $\log_{10}(p_1^\circ/p_2^\circ)$ depended only on the properties of the pure materials. The term X was solely a property of the binary mixture 1,3 and was only slightly "composition dependent" provided that the stationary phase was in large excess. Similarly, Y depended only on the binary mixture 2,3.

Now for most homologous series it was found empirically that equation (6) was true—

$$\log_{10} p_0 = A + Bn \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

where p_0 was the vapour pressure at $T°$ absolute of a member of the series containing n carbon atoms, and A and B were constants for a given series and temperature. Equation (6) was obeyed best if the first few members of the series were not included.

Gas chromatography was usually concerned with conditions in which component 3 was in large excess. In these circumstances X was approximately equal to $\log_{10}\gamma^\circ_{13}$, where γ°_{13} was the limiting value of the activity coefficient of component 1 in component 3 as $N_3 \rightarrow 1$ and with the pure components as the standard states. There were published values for $\log_{10}\gamma^\circ_{13}$ for a few dilute binary mixtures containing members of homologous series, *e.g.*, alcohols in water (Butler, J. A. V., Thomson, D.W., and MacLennon, W. H., *J. Chem. Soc.*, **1933**, 674) and pyridine bases in water (Andon, R. J. L., Cox, J. D., and Herington, E. F. G., *J. Chem. Soc.*, **1954**, 3188), from which it appeared that the results fitted equation (7).

$$X = C + Dn \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

Here C and D were constants for a given homologous series in a given solvent and n was the number of carbon atoms in the compound under consideration. This equation, like equation (6), was best obeyed if the first few members of the homologous series were not included.

The following conclusions were reached by combining expressions (1), (6) and (7)—

I (*a*)—The plot of $\log_{10} V_n$ against n for a given homologous series and stationary phase should yield a straight line. Here V_n was the retention volume of the compound containing n carbon atoms. This plot had been recognised as a useful one for some time (*e.g.*, by James, A. T., and Martin, A. J. P., *Biochem. J.*, **1952**, **50**, 679).

I (*b*)—Alternatively the plot of $\log_{10} V_{nr}$ against n for a given homologous series and stationary phase should yield a straight line. Here V_{nr} was the relative retention volume obtained by dividing the retention volume of the compound containing n carbon atoms by the retention volume of the compound r on the same column; r was kept constant for a given plot. This method of treating data had also been employed by A. T. James (*Biochem. J.*, **1952**, **52**, 242).

I (*c*)—The plot of $\log_{10} V_n$ against $\log_{10} p_n^\circ$ should yield a straight line. Here V_n was the retention volume of the pure component, which had a vapour pressure of p_n° at the temperature considered.

I (*d*)—The plot of $\log_{10} V_{nr}$ against $\log_{10} p_n^\circ$ or against $\log_{10}(p_n^\circ/p_r^\circ)$ should yield straight lines (see I (*b*) above for definition of V_{nr}).

Plots I (*c*) and I (*d*) were probably superior to plots I (*a*) and I (*b*), provided that accurate values for the vapour pressures of the components were available.

II (*a*)—To a first approximation, the plot of V_{nrI} against V_{nrII} should yield a straight line where V_{nrI} was the relative retention volume of component n with respect to component r in solvent I and V_{nrII} was the relative retention volume of component n with respect to component r in solvent II. Component r was kept constant for a given plot, but component r might not belong to the same homologous series as component n. The components n, $n+1$, $n+2$, etc., must belong to the same homologous series. These plots had frequently been used since A. T. James (*Biochem. J.*, 1952, **52**, 242) had reported linear relationships for amines in liquid paraffin and Lubrol MO. It followed from this treatment that, if n and r belonged to the same homologous series (*e.g.*, if both were straight-chain amines), then the slope of the plot of V_{nrI} against V_{nrII} should be approximately unity. As examples of this behaviour might be quoted the straight line produced for primary straight-chain amines by using relative retention volumes with respect to ethylamine (James, A. T., *Biochem. J.*, 1952, **52**, 242) and the straight line produced for aliphatics by using relative retention volumes with respect to *n*-pentane (James, A. T., *Research*, 1955, **8**, 8).

II (*b*)—To a second approximation the plot of $\log_{10} V_{nrI}$ against $\log_{10} V_{nrII}$ should give a straight line, where V_{nrI} and V_{nrII} were as defined in II (*a*) above. This method of plotting was in every way preferable to that given in II (*a*), for not only did it employ a better approximation but the points for successive members of a homologous series were nearly equally spaced along a line. This was a direct result of the conditions given by expressions (6) and (7).

Finally the ease of separation of two components could be considered by means of expression (1). If components 1 and 2 belonged to different chemical classes, then it was usually possible to choose an immobile phase (third component) such that $|X - Y|$ was not small. Hence, if 1 and 2 were of different chemical classes, it was usually fairly easy to separate them even if $\log_{10}(p_1^\circ/p_2^\circ)$ was numerically small (*i.e.*, if 1 and 2 had nearly the same boiling point). For example, it was easy to separate benzene (b.p. 80·1° C) from *cyclo*hexane (b.p. 80·8° C). Separation would, however, be difficult if $\log_{10}(p_1^\circ/p_2^\circ)$ was small and if at the same time a solvent could not be found that made $|X - Y|$ large. These circumstances would be expected to arise when components 1 and 2 were close-boiling isomers of very similar structures. Pairs of this type were *m*- and *p*-xylene (b.p. 139·1° C and 138·3° C, respectively), also β- and γ-picoline (b.p. 144·1° C and 145·4° C, respectively). In connection with these bases it might be noted that 2:6-lutidine (b.p. 144·0° C) could readily be separated from β- or γ-picoline by gas chromatography. This was understandable because the additional $>CH_2$ group in the lutidine made it easy to find immobile phases for which $|X - Y|$ was large enough to enable separation to be achieved.

The study of higher-boiling mixtures by gas chromatography would probably reveal many more pairs of close-boiling isomers of similar structure that were difficult to separate.

MR. G. A. VAUGHAN said that they were interested in the separation of the benzene homologues and he would like to refer, in this connection, to Dr. Martin's remarks on the increase of the separation factor with decrease of temperature. They had recently calculated the separation factor between the close-boiling isomers *m*- and *p*-xylene and had found it to be 1·021 at 140° C and 1·035 at 60° C. This implied that to separate these by three standard deviations a column efficiency of 20,800 plates would be needed at 140° C and only 7600 at 60° C. Hence, with their normal column of 1600 plates, a partial separation of 1·40 standard deviations would be expected at 60° C. This had been confirmed in practice when a separation of 1·38 standard deviations had been obtained, a change on the recorder trace from a single peak to one with a marked inflection.

MR. E. R. ADLARD asked Dr. Martin if he had yet proved that the gas-density balance gave a linear response for a binary mixture from 0 to 100 per cent. range of concentration.

DR. MARTIN replied that he had not made accurate measurements, but the response was linear within the accuracy of measurement. No doubt it was more linear at lower density differences.

DR. W. A. WISEMAN asked what effect changes of pressure drop across the column would have on efficiencies.

DR. MARTIN replied that changes in pressure drop across the column affected the efficiency primarily because of the change of rate of flow. With their conditions increase of pressure drop decreased efficiency. (Increasing total pressure at both ends would result in reducing efficiency, but it would not be a very large effect.)

THE VAPOUR-PHASE CHROMATOGRAPHIC ANALYSIS OF HYDROCARBON MIXTURES

MR. D. E. CHALKLEY said that vapour-phase chromatography was a method well suited to the analysis of complex hydrocarbon mixtures, either on its own or in conjunction with other physical methods such as infra-red and mass spectrometry. Techniques had been developed for the analysis of hydrocarbons ranging from methane (b.p. −161·5° C) to tri-*iso*propylbenzene (b.p. 240° C). He gave examples of their application both in the laboratory and for process monitoring of plant streams.

The C_1 to C_4 hydrocarbons had been the subject of a special investigation of the gas-liquid partition technique. Their relative retention volumes over a large number of stationary liquid phases had been recorded to illustrate the separations possible when different types of solubility forces were brought into play.

Finally, various aspects of the accurate quantitative analysis of hydrocarbon mixtures had been investigated by using a thermal-conductivity cell as a detector and hydrogen as a carrier gas. Results were compared with those by the mass spectrometer and some comparisons were made of the accuracy and sensitivity of the two methods.

TECHNIQUES USED IN A STUDY OF THE BORON AND SILICON HYDRIDES

MR. A. B. LITTLEWOOD outlined the more important general properties of the boron and silicon hydrides, and the methods used for their preparation and manipulation. The apparatus that was being used for their separation and analysis by gas chromatography was discussed with examples. An account was given of the method used for the identification of fractions from the chromatographic columns, in particular by effusiometry and pyrolysis.

Discussion

Mr. Freedman asked whether the author had found the thermal-conductivity cell sufficiently sensitive for his purpose.

Mr. Littlewood replied that, so far as the work had gone, it was sufficiently sensitive. There was no guarantee, however, that there were not further hydrides present in the chromatogram that were undetected by the cell. In order to look for such compounds, a more sensitive detector would be useful. Although the sensitivity of the cell could be raised by increasing the operating temperature of the wire, it was not possible to do this in the work described here because of the thermo-labile nature of the substances involved.

Adsorption and Partition Methods

Mr. C. S. G. Phillips gave a brief outline of work in his laboratory, where both partition and adsorption methods were used. Some details of apparatus were given and the use of glass thermal-conductivity cells and of the surface-potential detector was discussed.

Results obtained with partition chromatography were used to illustrate the value of fully corrected retention volumes, both as a basis for an absolute scheme of analysis and for thermodynamic calculations.

The paper was concluded with a general review of published work on adsorption gas chromatography (including both elution and displacement techniques) and a comparison of the displacement and elution methods.

A Rapid Chromatographic Method for the Determination of Bromine-inert Impurities in Ethylene*

Mr. N. H. Ray described his simplified method for the rapid determination of small amounts of non-olefinic impurities (other than carbon dioxide) in ethylene. The principle

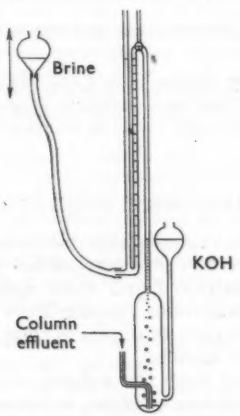

Fig. 1. Modified nitrometer

was as follows: a measured sample of the gas (25 or 50 ml) was carried in a current of pure carbon dioxide through a column, which was in two parts: the first was packed with a solid absorbent consisting of charcoal impregnated with bromine, which absorbed the ethylene and also retained the ethylene dibromide formed; the unabsorbed impurities passed into the second section of the column, packed with activated charcoal, where they were separated. The issuing gases were collected in a micro-nitrometer over potassium hydroxide solution and appeared in two or more discrete fractions, with a sufficient interval between them to permit separate measurement. The first fraction contained any hydrogen, oxygen, nitrogen, carbon monoxide and methane present in the sample, the second consisted of ethane only, and any higher paraffins appeared in subsequent fractions. The complete determination occupied only 15 to 20 minutes, and the smallest detectable concentration of any one impurity

* This paper was published in full in *The Analyst*, 1955, **80**, 853.

was 0·01 per cent. in a 50-ml sample. Sufficient bromine-impregnated charcoal could be accommodated in the column to last for about 30 analyses, and this reagent could be safely handled without any special precautions.

Discussion

Dr. M. M. Wirth said that they had been carrying out the same type of analysis and had also used Janak's method for measuring individual components of the mixture. However, they simply packed their chromatographic column with silica gel. The impurities to be measured, namely hydrogen, methane and ethane, all had lower retention volumes than ethylene, and therefore the large ethylene peak did not interfere with an accurate measurement of the impurities. They used a modified nitrometer as shown in Fig. 1. Its advantages were a constant head of liquid and a steady meniscus, which gave an early response to the rising gas bubbles.

Discussion on Katharometers as Recorders in Gas Chromatography

Dr. Keulemans opened the discussion by saying that his experience with katharometers was restricted to the metal-encased instruments, which they had found to operate very satisfactorily; but such instruments could not be used where materials that had corrosive effects on the metal parts had to be detected, as in Mr. A. F. Williams's work. But whether the cells were housed in metal or in glass, the problems remained much the same, although the magnitude of the effects might differ appreciably.

The ideal katharometer was designed to measure only the changes in concentration of a vapour in the gas passing through it; in practice fluctuations in the operating conditions would also produce a signal, which would manifest itself in either a "zero drift" or a wobble of the baseline. It was the task of the designer to construct an instrument in which these signals could be kept below a certain level, and the task of the user was to operate the instrument so that they remained below the desirable or detectable level. This level varied from one worker to another. Most of the undesired effects must be ascribed to the fact that in the cells heat was not only transported by conduction from the heated wire to the walls of the cell, but also by radiation, forced convection and conduction through the ends of the heated wire. With two completely identical cells, both operated under exactly the same conditions, the above-mentioned factors would produce no effect on the e.m.f. of the bridge of which the katharometer was a part. If one realised that generally the recorder deflections in actual vapour-phase chromatographic measurements were caused by a change in temperature of one of the heated wires by between 1° C and 0·001° C, it became evident that fluctuations in the operating conditions might produce considerable effects.

Before proceeding to discuss the Amsterdam design, he described their requirements. Gas chromatography was from the start considered to be an eminently suitable method for routine analysis. One of the problems was that, particularly for remote refineries in the Shell Group, an apparatus had to be designed that could be operated by personnel of very limited skill, which implied that a perfect baseline was essential. They had found that this could be achieved with an instrument consisting of a copper block in which two narrow parallel holes had been drilled, in the centre of which the platinum wires were suspended by means of leaf springs. Electrical leads through the block were made with glass seals. With this instrument thermostatic control to within $\pm 0{\cdot}1°$ C at temperatures up to 100° C and $\pm 0{\cdot}2°$ C for temperatures between 100° and 200° C was necessary. For a heating current of 200 mA, constancy to within ± 1 mA was required. This could be attained by using good storage batteries and periodic re-adjustment of the heating current by comparison with a standard-cell circuit. The gas flow during an analysis had to be kept constant to within $\pm$ 20 ml per hour at any flow rate, and this was easily accomplished with commercially available pressure regulators and control of the inlet and outlet pressures. In operation the katharometer was placed centrally in the coiled copper separating column with which it formed a single unit. Perfectly straight baselines were maintained for hours on end without any re-adjustment, which meant that the false signals were kept below 10 μV (0·25 per cent. of the full-scale deflection on a 2·5-mV recorder). This set-up had been described in a paper being presented to the IVth World Petroleum Congress.

Mr. A. F. Williams said that Dr. Keulemans had made considerable progress in his work on katharometers and had cleared up many of the points that had been worrying them. In their particular investigations they were using glass katharometers, and the technique

for their fabrication had been demonstrated during the afternoon. The katharometer formed part of a single unit with the chromatograph tube, the whole being heated by vapour in a metal jacket after the manner employed by James and Martin.

There were many factors that seemed to influence the stability of the system when they were working at high sensitivities. Experience had shown that above a certain temperature the katharometer might become unstable, and this temperature decreased as the sensitivity was increased and as the pressure on the outlet of the column was decreased.

Stability was also affected by the rate of return of reflux from the jacket to the boiler; this factor was related to the geometry of the apparatus.

The problem of "drift" of the zero shown on the recorder, which appeared to be associated with the effect of temperature change on the components of the bridge, was under investigation.

DR. KEULEMANS added that Mr. Williams, with his glass katharometer, had obtained a very satisfactory baseline below, say, 120 mA, and instability (wobble) at 150 mA. This might very well be ascribed to the difference in thermal-expansion coefficient between glass and platinum, as a consequence of which the heated wire was under insufficient tension at high temperatures, which would make the instrument very sensitive to vibration. With the metal katharometers the effect was just reversed, because platinum had the lower coefficient of expansion. One might get the impression that good results with vapour-phase chromatography could only be obtained with a perfect katharometer. In his view the glass instruments, which were cheap and fairly easy to construct, as might be seen from the instruments developed by Phillips and by Williams, would contribute much more to the development of this ingeneous chromatographic method than the expensive, more perfect instrument. The latter, however, was necessary for quantitative analysis on routine basis.

MR. D. HARVEY said that when using glass thermal-conductivity cells operating at around 240° C they found that good stability was not obtained unless the wires leading from the cells were protected from convection currents. Sufficient protection was afforded by a covering of asbestos-wool.

The body temperature of conductivity cells was important in qualitative as well as in quantitative work. For instance, they had found that, whereas the thermal conductivity of methanol vapour was lower than that of nitrogen at 20° C, at 100° C the reverse was true, and at some intermediate temperature they were equal. Similar changes had been noticed with other compounds, such as phenols and the lower aliphatic acids.

Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Preparation of Sample

Destruction of Organic Matter

In most instances, determination of the metallic constituents must be preceded by destruction of the organic matter by oxidation with nitric and sulphuric acids. Certain metals, however, require special treatment, which is described in the appropriate text.

APPARATUS—

This is shown in Fig. 1.

REAGENTS—

Distilled water—To be distilled from glass apparatus.
Nitric acid, sp.gr. 1·42.
Sulphuric acid, sp.gr. 1·84.
Hydrochloric acid, sp.gr. 1·18.
Ammonium oxalate solution, saturated.

PROCEDURE—

Into the 250-ml flask of the apparatus shown in Fig. 1 put a volume of the well mixed sample, the volume being of a suitable size for the particular determination or determinations. Acidify the sample with nitric acid, using about 5 ml in excess. Assemble the apparatus and evaporate the mixture to a volume of 5 to 10 ml. Add 2 to 3 ml of sulphuric acid and continue heating the solution until copious white fumes of sulphur trioxide are evolved. If the solution contains charred organic matter or shows any brown discoloration, carefully add 2 ml of nitric acid and again heat to fuming. If necessary, repeat the treatment with nitric acid until all organic matter is destroyed and the solution becomes colourless. To the cooled solution carefully add 10 ml of saturated ammonium oxalate solution, mix, and again heat to fuming.

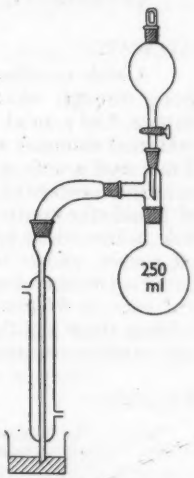

Fig. 1. Apparatus for wet oxidation of the sample

When carrying out the above operations, take care to avoid any sucking back of the distillate. Discard the distillate.

Cool the residue and add 10 ml of distilled water. Allow any insoluble matter to settle and then filter the liquid, receiving the filtrate in a 100-ml calibrated flask and retaining as much of the sediment as possible in the original reaction flask. Add 1 ml of hydrochloric acid to the residue in the flask, heat the solution gently, add 5 ml of hot distilled water and pass the liquid through the filter. Repeat the operation a second time, finally washing the flask and filter with hot distilled water. Cool the combined filtrates and dilute the solution to the mark with distilled water.

Prepare a blank solution in a similar manner, but without the effluent sample.

Methods for the Determination of Arsenic and Copper

Arsenic

Two methods are recommended for the determination of arsenic, namely, the Gutzeit method and the hypophosphite method. It was decided to omit the molybdenum-blue method, since sufficient general experience has not yet been obtained in this country.

Gutzeit method

PRINCIPLE OF METHOD—

After destruction of the organic matter, arsenic is reduced by means of nascent hydrogen to arsine, which is then passed through mercuric chloride paper and the stain produced is compared with standard arsenic stains.

RANGE—

For arsenic contents up to 10 μg.

APPLICABILITY—

The method is generally applicable.

APPARATUS—

A wide-mouthed bottle capable of holding about 120 ml is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6·5 mm (external diameter about 8 mm). It is drawn out at one end to a diameter of about 1 mm and a hole not less than 2 mm in diameter is blown in the side of the tube near the constricted part. When the bung is inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly flame-polished or ground smooth.

Two rubber bungs (about 25 × 25 mm), each with a hole central and true and exactly 6·5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively, the two bungs may be replaced by any suitable contrivance satisfying the conditions described under "Procedure."

REAGENTS—

NOTE—The reagents must be selected so as to be virtually free from arsenic. Such reagents can be purchased.

Hydrochloric acid, sp.gr. 1·18.

Stannous chloride solution—Dilute 60 ml of hydrochloric acid with 20 ml of distilled water. Add 20 g of tin, heat gently until the evolution of gas ceases and dilute to 100 ml, allowing the undissolved tin to remain in the solution. Add an equal volume of hydrochloric acid. Evaporate the solution to the original volume of 100 ml and filter it through a fine-grained filter-paper.

Stannated hydrochloric acid—Mix 1 ml of stannous chloride solution with 100 ml of hydrochloric acid.

Potassium iodide.

Zinc, granulated (special quality for Gutzeit test).

Standard arsenic solution A—Dissolve 0·132 g of arsenious oxide (≡100 mg of arsenic) in 50 ml of hydrochloric acid and dilute to 100 ml.

Dilute standard arsenic solution B—Dilute 1 ml of arsenic solution A to 100 ml. This solution shall be freshly prepared.

1 ml ≡ 10 μg of arsenic.

Lead acetate solution, 10 per cent. w/v.

Mercuric chloride paper—Smooth white filter-paper, not less than 25 mm in width, soaked in a saturated solution of mercuric chloride in distilled water, pressed to remove superfluous solution, and dried at about 60° C in the dark. The grade of the filter-paper shall be such that the weight in g per sq. metre shall be between 65 and 120; the thickness in millimetres of 400 papers shall be approximately equal, numerically, to the weight in g per sq. metre.

NOTE—Store the prepared paper in a stoppered bottle. Papers that are available commercially have been found to produce more uniform results than those prepared in the laboratory.

PROCEDURE—

Pack the glass tube of the apparatus lightly with cotton-wool that has been previously moistened with a solution of lead acetate and dried, so that the upper surface of the cotton-wool is not less than 25 mm below the top of the tube. Insert the upper end of the tube into the narrow end of one of the pair of rubber bungs, either to a depth of about 10 mm when the tube has a flame-polished end, or so that the ground end of the tube is flush with the larger end of the bung. Place a piece of mercuric chloride paper flat on the top of the bung, place the other bung over it and secure the two by means of a rubber band or spring clip in such a manner that the bores of the two bungs meet to form a true bore, 6·5 mm in diameter, interrupted by the diaphragm of mercuric chloride paper. This method of attaching the mercuric chloride paper may be replaced by any other method, provided that—

(*a*) the whole of the evolved gases pass through the paper,
(*b*) the portion of the paper in contact with the gas is a circle 6·5 mm in diameter, and
(*c*) the paper is protected from sunlight during the test.

Transfer an aliquot (preferably containing about 5 μg of arsenic) of the acid solution, prepared as described under "Destruction of Organic Matter," to the wide-mouthed bottle and dilute it to 50 ml. Add 10 ml of stannated hydrochloric acid, 1 g of potassium iodide and, finally, 10 g of granulated zinc. Immediately place the prepared glass tube in position. Allow the reaction to proceed for 40 minutes at a temperature not exceeding 40° C; adjust the temperature to obtain a regular, but not too violent, evolution of gas.

Compare the yellow stain produced on the mercuric chloride paper with a series of standard stains produced as described below.

The standard stains should be prepared at the same time as the test stain.

Carry out the procedure on the same volume of the blank solution referred to under "Destruction of Organic Matter." This stain should be negligible.

PREPARATION OF STANDARD STAINS—

Six sets of apparatus are required for the preparation of the standard stains. Charge each tube with cotton-wool, previously moistened with a solution of lead acetate and dried, and fix a piece of mercuric chloride paper as described under "Procedure." Into the wide-mouthed bottles measure quantities of dilute standard arsenic solution B varying from 0 to 1 ml in steps of 0·2 ml. Dilute the contents of each bottle to 50 ml, add 10 ml of stannated hydrochloric acid, 1 g of potassium iodide and 10 g of granulated zinc, and immediately place the prepared glass tube in position. Allow the reaction to proceed as described under "Procedure."

HYPOPHOSPHITE METHOD

PRINCIPLE OF METHOD—

The arsenic present is reduced to elementary arsenic. This is separated by filtration and determined iodimetrically.

RANGE—

For arsenic contents of 0·4 to 100 mg.

NOTE—For arsenic contents less than 0·4 mg and greater than 10 μg, the sample should be suitably diluted and the Gutzeit method applied.

APPLICABILITY—

The method is of wide applicability, although mercury, selenium and tellurium interfere.

REAGENTS—

Sodium arsenite solution, 0·1 N—Weigh 4·9455 g of arsenious oxide, previously dried at 105° C. Dissolve this in a solution of sodium hydroxide (2·5 g of sodium hydroxide pellets dissolved in about 50 ml of distilled water), with gentle warming. Cool the solution, add 66 ml of *N* hydrochloric acid and then 10 g of sodium bicarbonate. Transfer the solution to a 1-litre calibrated flask and dilute to the mark with distilled water.

Sodium arsenite solution, 0·01 N—Dilute the 0·1 *N* sodium arsenite solution, 1 in 10 with distilled water.

Iodine solution, 0·1 N.

Iodine solution, 0·01 N.

Hydrochloric acid, sp.gr. 1·18.

Sodium hypophosphite.

Ammonium chloride solution, 5 per cent. w/v.

Potassium iodide.

Sodium bicarbonate.

Filtering medium—Digest about twenty 11-cm filter-papers (Whatman No. 40) in 400 ml of distilled water containing 4 ml of hydrochloric acid and 20 ml of saturated bromine water on a water bath for about 4 hours. Store the pulped material in a glass-stoppered bottle. When preparing the filter-pad, wash it thoroughly with distilled water before using the filter.

PROCEDURE—

Transfer a suitable aliquot of the acid solution, prepared as described under "Destruction of Organic Matter," to a conical flask and dilute it to 75 ml with distilled water. Add 75 ml of hydrochloric acid and 4 g of sodium hypophosphite. Heat the solution for 5 minutes, but do not allow the temperature to rise above 50° C. Add a further 10 g of sodium hypophosphite, and close the mouth of the flask with a cork carrying a length of glass tubing, approximately 80 cm long and 0·5 cm internal diameter, to act as a reflux condenser. Bring the solution to boiling point and boil gently for 15 minutes. Cool and collect the precipitated arsenic on a pad of the previously prepared filter-pulp. If large amounts of arsenic are present, it is advisable to mix a little of the washed filter-pulp with the precipitate in the conical flask before filtering, in order to keep the precipitate in a finely divided condition, so that it will readily dissolve in the iodine solution used for the titration. Wash the precipitate on the filter-pad with an acid solution of sodium hypophosphite, prepared by adding 2 g of sodium hypophosphite to 100 ml of a solution of diluted hydrochloric acid (1 + 3). Finally wash the precipitate with ammonium chloride solution to remove sodium hypophosphite.

Transfer the precipitate and paper pulp to a 16-oz wide-mouthed glass-stoppered bottle containing about 50 ml of distilled water. Disintegrate the precipitate and paper pulp, and add 2 g of potassium iodide and 2 g of sodium bicarbonate. Then add 0·1 *N* iodine solution (or 0·01 *N*, the particular strength of iodine solution to be used depending upon the amount of precipitate) sufficient to react with the arsenic and to give about 10 ml in excess. Shake the contents of the bottle thoroughly to dissolve the arsenic. Remove the stopper, rinsing it with distilled water, and immediately titrate the excess of iodine with either 0·1 *N* or 0·01 *N* sodium arsenite solution, according to the concentration of the iodine solution used. The end-point, *i.e.*, the change from the yellow of the iodine solution to the white of the oxycellulose pulp, is quite sharp.

$$\frac{\text{Volume (ml) of 0·1 } N \text{ iodine solution} \times 0{\cdot}00149 \times 10^6}{\text{Volume (ml) of original sample}} = \text{Arsenicc ontent (mg per litre).}$$

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Copper

PRINCIPLE OF METHOD—

After destruction of the organic matter, the copper is extracted from alkaline citrate solution as its diethyldithiocarbamate and then determined colorimetrically.

RANGE—

For copper contents up to 50 μg.

APPLICABILITY—

The method is of wide applicability, although bismuth interferes.

REAGENTS—

NOTE—Water distilled from glass apparatus must be used throughout.

Sodium diethyldithiocarbamate solution—Dissolve 1 g of sodium diethyldithiocarbamate in 100 ml of water and filter the solution.

EDTA - citrate solution—Dissolve 20 g of ammonium citrate and 5 g of disodium ethylenediaminetetra-acetate (EDTA) in water and dilute to 100 ml with water.

Standard copper solution—Dissolve 0·200 g of pure copper wire in 15 ml of diluted nitric acid (1 +4), boil to expel fumes, cool and dilute to 200 ml with water. Dilute 10 ml of this solution to 1 litre as required.

1 ml $\equiv$ 10 μg of copper.

Ammonium hydroxide, sp.gr. 0·880.

Cresol red indicator solution, 0·04 *per cent. in ethanol* (*I.M.S.*).

Carbon tetrachloride.

PROCEDURE—

Transfer 25 to 50 ml of the acid solution, prepared as described under "Destruction of Organic Matter" and containing not more than 50 μg of copper, to a separating funnel. Add 10 ml of EDTA - citrate solution and 2 drops of cresol red indicator solution. Add ammonium hydroxide until the solution is purple - red, cool and add 1 ml of sodium diethyldithiocarbamate solution. Add 10 ml of carbon tetrachloride and shake the funnel for 2 minutes. Allow the layers to separate; then remove the lower layer and filter it through a dry filter-paper into a 25-ml calibrated flask. Extract the aqueous layer with further 5-ml portions of carbon tetrachloride until no further colour is extracted. Filter the extracts as before through the same filter-paper and dilute to the mark with carbon tetrachloride. Measure the optical density in a spectrophotometer or an absorptiometer, using a wavelength of 4320 A in a spectrophotometer, or a suitable blue filter if an absorptiometer is used. Read the number of micrograms of copper equivalent to the observed optical density of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of copper in the sample.

Establish the calibration graph as follows—

To 50 ml of glass-distilled water containing 1 ml of sulphuric acid, add appropriate amounts of standard copper solution to cover the range 5 to 50 μg, and proceed as described above. Construct a graph relating optical densities to micrograms of copper.

INTERFERENCE OF BISMUTH—

Bismuth is also extracted by the carbamate reagent under the conditions described above. If bismuth is present, the determination should be repeated, with potassium cyanide solution added before the sodium diethyldithiocarbamate solution, and the second reading should be subtracted from the first to give the optical density due to the copper.

Notes

IMPROVED CONDITIONS FOR THE SODIUM PHENOXIDE - SODIUM HYPOCHLORITE METHOD FOR THE DETERMINATION OF AMMONIA

In his method for the determination of ammonia in natural waters, Riley[1] makes use of the blue colour produced by the sodium phenoxide - sodium hypochlorite reaction with the ammonia. To the test solution are added sodium phenoxide solution (phenol + sodium hydroxide), sodium hypochlorite solution and manganese sulphate in that order. After dilution to 25 ml with distilled water and mixing, the solution is heated to 70° C for 45 minutes. The solution is then cooled and the absorbancy is measured at 625 mμ after standing for a further 10 minutes. Beer's law is followed for amounts up to about 50 μg, but the curve is reproducible up to 150 μg.

During routine checking of the absorptiometric procedure on aqueous ammonium chloride solutions, with the Spekker absorptiometer, Calorex H503 heat filters and Ilford No. 607 filters, it was noted that wide variations occurred between successive calibration curves. Factors (*i.e.*, micrograms of nitrogen equivalent to unit absorbancy) ranging from 61 to 75 were obtained over a period of a week for a volume of 25 ml and a 2-cm cell. As the sodium phenoxide had to be renewed at least every 2 days because of darkening, so necessitating re-calibration, this was a hindrance to the usefulness of the method. It was found that, if a stock solution of phenol in industrial methylated spirit was prepared, together with a corresponding solution of sodium hydroxide in water, portions could be mixed as required, thus avoiding the variations arising when relatively small quantities of phenol were weighed. Both stock solutions were stored in the refrigerator. By using this procedure, factors ranging from 53·4 to 54·5 were obtained over a period of 6 days, the sodium phenoxide being freshly made up each day. In a second test the range was 53·6 to 56·2 over a period of 10 days. Further solutions gave factors of 54·7 and 55·2 when freshly prepared and 55·0 after storage in the refrigerator for 9 months.

Apart from the improved method of preparing the sodium phenoxide, which should reduce the necessity for frequent re-calibration, there appeared also to be some gain in sensitivity, which resulted from the introduction of the industrial methylated spirit. This was confirmed by comparing the net absorbancy due to 30 μg of nitrogen (as ammonium chloride) with and without the addition of industrial methylated spirit. These were 0·50 for the aqueous sodium phenoxide compared with 0·56 for another aliquot of the same sodium phenoxide to which 0·4 ml of industrial methylated spirit had been added.

Further tests indicated that greater enhancement of the blue colour was obtained if *iso*-propanol, *n*-butanol or acetone were used in place of industrial methylated spirit, but that some diminution occurred with methanol. Of these substances acetone stood out as exceptional. Not only did it give the greatest absorbancy, but the blue colour developed rapidly in the cold and reached its maximum absorbancy after 20 minutes at room temperature. Heating to 70° C for 45 minutes, as specified in Riley's method, caused fading, although the colour could be largely regenerated by shaking the solution. The optimum amount of acetone was between 0·08 and 0·20 ml, the absorbancy being reduced if amounts outside this range were used.

The rapidity with which the colour developed suggested that the addition of manganese sulphate as a catalyst might be unnecessary. Calibrations of the Spekker absorptiometer were carried out with and without manganese sulphate but including 0·15 ml of acetone, and the results in Table I show that the manganese sulphate may be omitted. The volume of solution was 25 ml and 2-cm cells were used.

TABLE I

CALIBRATION FIGURES WITH AND WITHOUT MANGANESE SULPHATE

Nitrogen (as ammonium chloride), μg	4	8	12	16	20
Absorbancy with manganese sulphate present ..	0·113	0·230	0·340	0·455	0·570
Absorbancy with manganese sulphate absent ..	0·108	0·230	0·345	0·460	0·580

Further calibration experiments indicated that the curves are linear up to at least 80 μg of nitrogen for a volume of 25 ml and a 1-cm cell and up to 200 μg for a volume of 50 ml and a 1-cm cell. In the latter case, however, it is necessary to dilute the solution to 50 ml before the standing period.

Method

Reagent—

Sodium phenoxide reagent—Dissolve 62·5 g of analytical-reagent grade phenol in industrial methylated spirit, add 18·5 ml of acetone and dilute to 100 ml with industrial methylated spirit. Dissolve 27 g of sodium hydroxide in distilled water and dilute to 100 ml with distilled water. Store the solutions in the refrigerator. The sodium phenoxide reagent is prepared as required by mixing 20-ml portions of the above solutions and diluting to 100 ml with water.

Procedure—

Dilute the test solution to 10 ml with distilled water and add 4 ml of sodium phenoxide reagent. After mixing, add 3 ml of sodium hypochlorite solution (0·9 per cent. of available chlorine). Mix and dilute to 25 ml with distilled water. Mix the solution and set aside for 20 minutes at room temperature. Measure the absorbancy on the Spekker absorptiometer, using Calorex H503 heat filters, Ilford No. 607 filters and a 2-cm cell. Carry out a blank determination on the reagents, substituting 10 ml of distilled water for the test solution.

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THE TITRIMETRIC DETERMINATION OF CADMIUM WITH POTASSIUM FERROCYANIDE

The semi-micro technique of Frost[1] for zinc, as described by Milton and Waters,[2] involves the use of *o*-dianisidine as internal indicator. The zinc solution is buffered with ammonium chloride and hydrochloric acid and titrated at boiling temperature. It was found that under these conditions the colour development of the indicator was not wholly reliable and that there was a tendency to interference, owing to the formation of ferric ferrocyanide in the hot solution. Experiments showed that the chloride buffer could be replaced either by ammonium sulphate - sulphuric acid or by ammonium acetate - acetic acid. The colour was then obtained with great clarity and the titration could be performed at room temperature. Frost[1] and Milton and Waters[2] state that addition of ferricyanide should be made after commencing the titration and I found that the results were best when this addition was delayed until just before the end-point, the indicator being added after the ferricyanide. I was then able to apply the method successfully to the determination of cadmium. Belcher, Nutten and Stephen[3] describe 3:3′-dimethylnaphthidine as an internal indicator in this titration and note the high stability of this compound and the sharpness of the end-point. The findings of Belcher *et al.*[3] were confirmed, but *o*-dianisidine was found to give an extremely vivid vermilion colour in the acetate-buffered solution and, although use of acetic acid enhanced the colour and end-point of the 3:3′-dimethylnaphthidine, *o*-dianisidine was nevertheless preferred. Difficulty due to the false end-point, noted by Richardson and Bryson[4] in the titration of zinc, was not encountered. Stirring was found to be a very important factor and use of a magnetic stirrer greatly enhances precision.

Experimental

The following description refers throughout to the determination of cadmium, but is equally applicable to zinc.

Reagents—

o-Dianisidine indicator—Dissolve 0·1 g of *o*-dianisidine in 25 ml of 2·5 per cent. w/v sulphuric acid and dilute to 100 ml with water.

Potassium ferrocyanide—A 0·05 *N* aqueous solution prepared from the AnalaR reagent.

Potassium ferricyanide—A 2 per cent. w/v aqueous solution prepared from the AnalaR reagent.

Ammonium acetate.

Acetic acid, glacial.

PROCEDURE—

The sample, preferably as sulphate, should be contained in 30 to 50 ml of solution. Add 10 g of ammonium acetate and 10 ml of glacial acetic acid, the solution being well stirred. Use a semi-micro burette for the titration, stirring the sample vigorously the whole time. After 0·5 ml of titrant has been added a precipitate forms. Add two drops of ferricyanide solution, and then 10 drops of indicator. The vivid orange-red colour develops very rapidly and the titration should be continued to the pale green end-point. Perform a duplicate titration, this time delaying the addition of ferricyanide and indicator until 0·5 ml before the end-point previously obtained.

RESULTS

A solution was prepared from AnalaR cadmium sulphate, $3CdSO_4.8H_2O$, to contain 2·00 mg of cadmium per ml. This figure was confirmed by gravimetric analysis, 8-hydroxyquinoline being used, and also by passage of aliquots through an ion-exchange column containing Zeo-Karb 225 in the hydrogen form. The effluent acid was titrated and the results by the two methods agreed to within 0·001 g per litre. This solution was used to standardise the potassium ferrocyanide solution and to prepare samples that were analysed by three different operators with the following results—

Cadmium present, mg	.. ..	40·0	40·0	20·0	20·0	10·0	10·0	21·0	21·0	12·0	12·0
Cadmium found	Operator A, mg	40·0	39·5	20·0	20·0	10·1	10·0	21·0	21·0	11·9	12·0
	Operator B, mg	39·5	39·5	19·8	19·8	10·0	10·0	20·8	21·0	11·8	11·8
	Operator C, mg	40	40	19·7	20·0	10·0	10·0	21·0	21·0	11·9	12·0

With one exception no positive errors were found, but negative errors up to 1·6 per cent. occurred.

INTERFERENCES

Any substances tending to form ferrocyanide complexes should be absent, particularly copper and iron, also reducing agents, oxidising agents, nitrites and phosphates. Chloride and nitrate should be reduced to a minimum.

Under the conditions of these experiments quantities greater than 40 mg may give difficulty owing to the quantity of precipitate, and it is preferable to take an aliquot of the solution and dilute. The colour change is fully reversible at the end-point and, if this point is accidentally passed, the original orange-red colour may be restored by adding a small measured quantity of standard cadmium solution. Titrations should always be performed under exactly the same conditions used to standardise the ferrocyanide solution; for example, the same buffer should be used throughout. The ferrocyanide and ferricyanide solutions are somewhat unstable and should not be used more than 7 days after preparation or if their colour alters or sedimentation occurs. The sample to be titrated should not be set aside for more than 10 minutes after buffering before commencing the titration.

The complete determination may be carried out in a few minutes and requires no complicated apparatus.

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APPLIED ION EXCHANGE DEPARTMENT
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ANALYSIS OF TECHNICAL MONOGLYCERIDES WITH POTASSIUM PERIODATE

THE reagent commonly employed in the analysis of technical monoglycerides is a solution of periodic acid[1,2,3] or sodium periodate[4] in acetic acid. The sample is usually dissolved in chloroform and after the extraction of the uncombined glycerol with water, the aqueous and chloroform solutions are analysed separately.[3] Recently Pedersen[5] and Kruty, Segur and Miner[6] suggested dispensing with the use of acetic acid and determining the excess of periodate with arsenite instead of with sodium thiosulphate to increase the accuracy of the titration. Another modification introduced by Kruty *et al.* is the determination of glycerol with an aqueous solution of periodic acid without separating the aqueous and chloroform layers.

However, in the absence of acetic acid the oxidation of monoglycerides by periodate must be carried out at elevated temperatures, which might occasionally lead to undesirable side-reactions. Further, the solution of periodic acid in methanol employed by Kruty *et al.* is unstable and a disadvantage common to most methods referred to arises from the use of periodic acid, which is hygroscopic and often contains impurities. A modified procedure is therefore proposed in which acetic acid is retained, but periodic acid is replaced by potassium periodate available commercially in a high degree of purity. The oxidising reagent consists of potassium periodate dissolved in a small amount of 10 *N* sulphuric acid and diluted with acetic acid. A mixture of pyridine and acetic acid is added to the chloroform used for dissolving monoglycerides, so preventing their decomposition by sulphuric acid and ensuring solution of uncombined glycerol. An aqueous solution of potassium periodate is used for determining glycerol in the manner suggested by Kruty *et al.*

METHOD

REAGENTS—

Potassium periodate in sulphuric acid - acetic acid mixture—Dissolve 2·8 g of analytical-reagent grade potassium periodate with gentle warming in 50 ml of 10 *N* sulphuric acid (1 volume of concentrated sulphuric acid + 3 volumes of water). When the solution is cold, add 975 ml of glacial acetic acid. Store in a glass-stoppered brown bottle.

Potassium periodate solution—Dissolve 3·5 g of analytical-reagent grade potassium periodate with warming in 1 litre of water. Store in a glass-stoppered brown bottle.

Solvent for monoglycerides and blanks—Add 40 ml of pyridine - acetic acid mixture (1 + 1) to 160 ml of chloroform, B.P.

Sodium thiosulphate, 0·1 N solution, accurately standardised.

Potassium iodide solution, approximately N.

Sulphuric acid solution, approximately 2 N.

Starch indicator, 1 per cent. solution.

PROCEDURE—

The size of the sample used for analysis is governed by the monoglyceride and glycerol content, which must be approximately known. The amount of periodate employed in each test is sufficient to oxidise 0·16 g of monoglyceride or 0·022 g of glycerol. As the proportions of these components in the sample are not related, a preliminary experiment might be required.

Dissolve the sample in about 50 ml of chloroform - pyridine - acetic acid mixture and dilute the solution to 100 ml with the solvent.

In the determination of monoglycerides, put 25 ml of the above solution by pipette into a 500-ml glass-stoppered flask and then add 50 ml of the potassium periodate in sulphuric acid - acetic acid. Set the flask aside for 30 minutes in the dark, and then add 20 ml of potassium iodide solution and 100 ml of water; titrate the liberated iodine with sodium thiosulphate, using starch as indicator. Carry out a similar procedure for the blank, but using the solvent mixture in place of the monoglyceride solution.

In the determination of glycerol, place 25 ml of the sample solution by pipette into a 500-ml glass-stoppered flask, and then add 100 ml of water, swirling the mixture several times. Add 25 ml of aqueous potassium periodate solution and set the flask aside for 30 minutes in the dark, with occasional swirling. Add 20 ml of potassium iodide and 25 ml of 2 *N* sulphuric acid, and titrate the liberated iodine with sodium thiosulphate. Use 25 ml of the solvent mixture in the blank determination.

From the two titrations the percentages of monoglyceride and glycerol are calculated as follows—

$$\text{Monoglyceride, per cent.} = \frac{(T - T_1) \times N \times 0{\cdot}05\,M}{W}.$$

$$\text{Glycerol, per cent.} = \frac{T_1 \times N \times 2{\cdot}302}{W}.$$

T = titration of blank − titration of sample (monoglyceride + glycerol),
T_1 = titration of blank − titration of sample (glycerol),
N = normality of sodium thiosulphate,
W = weight of sample in the aliquot taken for test, and
M = molecular weight of monoglyceride.

Results

The monoglycerides examined in this work included technical monostearate, linseed-oil monoglyceride and a sample of monostearin prepared in the laboratory by the method of Fischer, Bergman and Bärwind.[7] They were analysed by the method of Pohle and Mehlenbacher[3] and by the modified procedure described in this Note. The reproducibility of the results is approximately ± 0·5 per cent. for monoglycerides and ± 0·2 per cent. for glycerol. A comparison of results obtained with the two methods is given in Table I.

Table I

Determination of monoglycerides and uncombined glycerol by (*a*) Pohle and Mehlenbacher's method and (*b*) potassium periodate method

Sample	Monoglyceride		Uncombined glycerol	
	Method (*a*), %	Method (*b*), %	Method (*a*), %	Method (*b*), %
Technical monostearate	38·45	38·40	6·74	6·94
	38·79	39·81	6·99	7·19
	39·67	40·02	7·09	7·35
Linseed-oil monoglycerides ..	39·59	40·02	4·88	5·04
	40·16	40·13	5·11	5·21
	40·89	40·90	5·16	5·32
Monostearin	98·73	98·82	—	—
	98·90	99·28	—	—
	99·41	99·65	—	—

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L. Hartman

April 27th, 1955

Ministry of Agriculture, Fisheries and Food

STATUTORY INSTRUMENTS*

1955—No. 1898 (C. 18). The Food and Drugs Amendment Act, 1954 (Appointed Day) Order, 1955. Price 2d.

This Order brings the Food and Drugs Amendment Act, 1954, into operation on January 1st, 1956 (with the exception of section 28, which came into operation on November 25th, 1954.)

The Food and Drugs Act, 1955, will come into operation as a consequence of the coming into operation of the 1954 Act, on the same day and immediately after the 1954 Act comes into operation. The 1955 Act is a consolidation measure; it repeals the 1954 Act and consolidates it together with the Food and Drugs Act, 1938, the Food and Drugs (Milk, Dairies and Artificial Cream) Act, 1950, and certain other enactments amending and supplementing Part V of the 1938 Act in relation to slaughterhouses and knackers' yards.

1955—No. 1899. The Food Standards (Butter and Margarine) Regulations, 1955. Price 2d.

These Regulations, which came into operation on January 1st, 1956, should be read with the Food Standards (General Provisions) Order, 1944, as amended (now having effect as if contained in regulations made under section 4 of the Food and Drugs Act, 1955). They prescribe standards for butter and margarine similar to those previously contained in section 32 of the Food and Drugs Act, 1938.

1955—No. 1900. The Labelling of Food (Amendment) Regulations, 1955. Price 3d.

These Regulations, which came into operation on January 1st, 1956, apply in England and Wales only. They amend the Labelling of Food Order, 1953, as amended (which Order has effect in England and Wales as if contained in regulations under section 7 of the Food and Drugs Act, 1955), to re-enact with modifications certain requirements of the repealed sections 32 (2) and 33 of the Food and Drugs Act, 1938, relating to the labelling, marking and advertising of margarine and margarine-cheese.

The new definition of "margarine" contained in The Food Standards (Butter and Margarine) Regulations, 1955 (above), is repeated in these Regulations.

The Regulations also substitute the description "imitation cream" for "synthetic cream" in item 3 of Table A in the First Schedule to the principal Order.

1955—No. 1901. The Mineral Oil in Food (Amendment) Regulations, 1955. Price 2d.

These Regulations, which came into operation on January 1st, 1956, amend the Mineral Oil in Food Order, 1949, as amended, which, by virtue of section 136 (2) and Part I of the Twelfth Schedule to the Food and Drugs Act, 1955, is continued in force in England and Wales as if contained in regulations made under section 4 of that Act.

These amending Regulations, having regard to the inclusion of chewing compounds in the definition of "food" in the 1955 Act, provide that the prohibition in the principal Order relating to mineral oil in food shall not apply in relation to chewing compounds which contain not more than 12·5 parts by weight of microcrystalline wax per 100 parts by weight of chewing compound and otherwise contain no mineral oil.

A standard of purity for microcrystalline wax is contained in the definitions in these Regulations.

FOOD STANDARDS COMMITTEE

Recommendations Relating to the Use of Colouring Matters in Foods

The Minister of Agriculture, Fisheries and Food has approved for publication a Supplementary Report presented to the Food Standards Committee by their Preservatives Sub-Committee, making recommendations about the use of colouring matters in foods.

Any representations should be addressed to the Assistant Secretary, Food Standards and Hygiene Division, Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1, to arrive not later than January 31st, 1956.

Copies of this Supplementary Report may be obtained from H.M. Stationery Office, price 9d. (plus postage).

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

British Standards Institution

AMENDMENT SLIPS*

PRINTED slips bearing amendments to British Standards have been issued by the Institution, as follows—

PD 2329—Amendment No. 1 (October, 1955) to B.S. 391:1949. Tung Oil.
PD 2334—Amendment No. 2 (November, 1955) to B.S. 2005:1953. Glossary of Terms Applicable to Fillings and Stuffings.
PD 2345—Amendment No. 2 (November, 1955) to B.S. 846:1952. Burettes and Bulb Burettes.
PD 2346—Amendment No. 1 (November, 1955) to B.S. 1428:Part E1:1953. Crucibles for Microchemical Analysis.
PD 2348—Amendment No. 2 (November, 1955) to B.S. 604:1952. Graduated Measuring Cylinders.
PD 2354—Amendment No. 1 (December, 1955) to B.S. 1911:1953. Genuine Hard Soap.
PD 2355—Amendment No. 1 (December, 1955) to B.S. 1912:1953. Soap Flakes.

Book Reviews

THE EXTRA PHARMACOPOEIA (MARTINDALE). Published by direction of the Council of the Pharmaceutical Society of Great Britain. Twenty-third Edition. In two Volumes. Volume II. Pp. xxxii + 1501. London: The Pharmaceutical Press. 1955. Price 57s. 6d.

The first edition of "Martindale," published in 1883, consisted of a single volume of 313 pages. It was divided into two volumes at the fifteenth edition in 1912 and until recent years the second volume was appreciably the smaller, but now, for the first time, it has become slightly larger. It may be explained that, broadly, Volume I is concerned with descriptions of drugs and their preparations together with notes and references to the medical literature on their usage, while Volume II is devoted to descriptions of practical procedures in analytical chemistry, biochemistry, bacteriology, pathology, nutrition and clinical methods.

The present work opens with 446 pages of Analytical Addenda to Materia Medica, all clearly printed in closely set small type. Besides embracing significant data additional to that embodied in the British Pharmacopoeia, 1953, and the British Pharmaceutical Codex, 1949 and 1954, it also includes a number of assays and standards of the United States Pharmacopoeia XIV and the American National Formulary IX, while some of the important changes in standards made in the U.S.P. XV (1955) and the N.F. X are listed in the Preface. This is the largest and most important section of the book, and comparison with the same part of the twenty-second edition (1943) shows that every monograph has been revised and the old Latin titles discarded. Hundreds of *working* abstracts of analytical methods are given, in every case with the appropriate reference to the original literature, many to papers in *The Analyst*.

The majority of the remaining sections have been written for this edition by specialists whose names are listed in the Preface. In order to give some idea of the enormous field of knowledge covered by this book it may be helpful to mention the titles of the sections, at the same time again reminding the reader of the small but clearly printed type employed throughout. Indeed, certain sections may well be regarded as sizable books of practical reference on their own account. Enumerating, after the Analytical Addenda section, we have: Hydrogen-ion Concentration; Oxidation - Reduction Potentials; Polarographic Analysis; Indicators; Ion-exchange Resins; Titration in Non-aqueous Media; Microchemical Analysis; Chromatographic Analysis; Analytical Absorption Spectrophotometry; Fluorimetric Analysis; Microbiological Assay of Vitamins; Food Analysis and Food Law (94 pages); Water Analysis; Recognition of Organic Substances; Nomenclature of Organic Compounds; Relation between Chemical Constitution and Therapeutic Effect; Compressed Tablets; Bacteriological and Clinical Notes with Reference to Special Diseases and Culture Media for Bacteriological Investigation (110 pages); Sterilisation; Disinfectants; Radiotherapy; X-Ray Diagnosis; Electrotherapy; Diathermy; Radiant Energy; British Spas; Nutrition; Vitamins; Haematology and Clinical Biochemistry; Proprietary Medicines. The Index comprises 67 pages and contains about 9400 references. In all this there is material of value to almost every analyst, whatever his speciality may be. The Contribution on Food Analysis and Food Law, by T. McLachlan and F. A. Lyne, justifies the comment already made to the effect that some sections are almost textbooks in themselves. Here we have a wealth of authoritative

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

practical information most conveniently arranged, and one observes that even the Lane and Eynon sugar tables are included. A particularly attractive feature of this section is the close juxtaposition of the details for carrying out the laboratory work and the legal considerations governing the interpretation of results. So far as the reviewer could ascertain, every legal standard relating to food with full references to all Regulations and Orders is given.

The ever-increasing importance of tablets for the administration of medicine has prompted the introduction of a section new to this edition, entitled "Compressed Tablets," in which a well known expert, A. Nutter Smith, summarises the essential features of this important subject and includes the methods of preparation and standardisation of physical properties of the various kinds of tablets.

There is only space to comment on one other section, namely, Haematology and Clinical Biochemistry. Actually, Haematology is presented as a separate section, but for the purposes of review may be conveniently linked to the one that follows. Valuable practical notes on the chemical analysis of body fluids have always been a prominent feature of "Martindale" and now the 78-page section of the previous edition is replaced by a thorough-going fully documented practical discourse written by D. N. Baron, E. T. Knudsen and C. F. Rose, comprising 51 pages on Haematology and 298 pages dealing with Clinical Biochemistry.

The work of arranging all this information has been undertaken by the Staff of the Scientific Publications Department of the Pharmaceutical Society of Great Britain under the Editorship of K. R. Capper and Assistant Editorship of S. C. Jolly, and to all of them the analyst has reason to be grateful. The book is well produced and abundantly worth its moderate price.

N. L. ALLPORT

ANALYSIS OF INSECTICIDES AND ACARICIDES. By FRANCIS A. GUNTHER and ROGER C. BLINN. Pp. xi + 696. New York and London: Interscience Publishers Inc. Price $14.00; 112s.

One of the principal developments in agricultural science in post-war years lies in the field of insecticides and fungicides. Whereas before 1938 the number of such compounds in common use in agriculture and horticulture could probably be counted on the fingers of two hands, the number of such compounds developed since that time, many indeed of which are now in common use, must run well into three figures.

The use of these new compounds is now very widespread and, although they are usually selected on account of their low toxicity to man, nevertheless nearly all of them, if ingested in sufficient quantity, are harmful. Their use on a scale undreamed of in 1938 constitutes a new hazard to man's food and thereby immediately produces new problems for the analyst in the detection and determination of residues remaining on crops at the time of sale to the public.

With these facts in mind, all analysts concerned with the examination of foodstuffs will welcome the present volume, which deals not only with the determination of such residues, but also with the examination of commercial insecticides and acaricides themselves.

The book is divided into a number of sections, the most important of which deal with: (1) sampling—a matter of considerable importance when it is realised that of necessity residues, the amount of which depends on many factors, are bound to be unevenly distributed; (2) preliminary extraction of the residues; (3) methods for "cleaning up" the extracts in order to remove interfering substances before specific tests for the compound that is being sought are applied; and (4) analytical procedures.

This last part of the work occupies more than half the volume and the majority of the now known insecticides and acaricides are considered in some detail.

In each instance the physical properties of the compound are given and this is followed by methods for the examination of the commercial insecticide as it is sold to the farmer. Finally methods are given for the examination of the "cleared extracts" obtained as described in section (3).

In most instances more than one method of analysis is given for a particular compound. Some of these methods are those now officially recognised by standardising bodies in different parts of the world, whereas others are new and have yet to be rigorously examined by collaborative investigations.

In devising methods of analysis both for the commercial insecticides and for residues, full advantage has been taken of recent developments in analytical chemistry and, apart from colorimetric methods, the methods include bio-assays, physical methods and radioactive-tracer techniques.

At the end of the book detailed ultra-violet and infra-red spectra are given for such compounds as lend themselves by virtue of their molecular structure to such examination.

It is not possible in a review of this nature to criticise the methods recommended by the authors, which have as yet not been generally accepted. No doubt some criticisms will appear in the literature in due course.

It is unfortunate that no mention is made of the examination of mercury compounds, but this may well be due to the fact that such compounds are apparently not regarded with any great favour in America.

Finally, the authors are to be congratulated on producing a book that is full of valuable information, much of which is clearly based on their own practical experience and which should be of considerable value to all analysts working in this field. J. HUBERT HAMENCE

Publications Received

BENTLEY AND DRIVER'S TEXT-BOOK OF PHARMACEUTICAL CHEMISTRY. Revised by JOHN EDMUND DRIVER, M.A., Ph.D., M.Sc., F.R.I.C. Sixth Edition. Pp. viii + 751. Oxford University Press. London: Cumberlege. 1955. Price 55s.

ANALYSE QUALITATIVE MINÉRALE. By PAUL-E. WENGER, Dr. ès Sc., DENYS MONNIER, Dr. ès Sc., and YVONNE RUSCONI. Pp. xviii + 315. Geneva: Georg & Cie., S.A. 1955. Price Sw.fr. 25.

MIKROSKOPISCHE UNTERSUCHUNG PFLANZLICHER NAHRUNGS-UND GENUSSMITTEL. By the late Dr. GUSTAV GASSNER, revised by Dr. FRIEDRICH BOTHE. Third Edition. Pp. xvi + 400. Stuttgart: Gustav Fischer Verlag. 1955. Price DM 33.

THE ANALYSIS OF MINERALS AND ORES OF THE RARER ELEMENTS. By the late W. R. SCHOELLER, Ph.D., F.R.I.C., and A. R. POWELL, F.I.M., F.R.I.C., F.R.S. Pp. xvi + 408. Third Edition. London: Charles Griffin & Company Limited. 1955. Price 60s.

EXPERIMENTAL DESIGN AND ITS STATISTICAL BASIS. By D. J. FINNEY, M.A., Sc.D., F.R.S. Pp. xii + 169. London: Cambridge University Press. 1955. Price 30s.

THE NATIONAL FORMULARY. Tenth Edition. Pp. xliv. + 867. Washington, D.C.: American Pharmaceutical Association. 1955. $9.00 (in U.S.A.); $10.00 (elsewhere).

Portraits of Past Presidents

THREE further photogravure portraits in the series of Portraits of Past Presidents are now in preparation, those of Mr. Lewis Eynon (President: 1947–1948), Mr. George Taylor, O.B.E. (President: 1949–1950), and Dr. J. R. Nicholls, C.B.E. (President: 1951–1952). Members of the Society and subscribers to *The Analyst* who wish to receive gratis copies of these three portraits should apply to the Editor, *The Analyst*, 7–8 Idol Lane, London, E.C.3, before February 29th, 1956.